New York (State) Dept. of agriculture & markets
Annual report. v. 3

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STATE OF NEW YORK

THIRTY-EIGHTH ANNUAL REPORT

OF THE

New York Agricultural Experiment Station.

(GENEVA, ONTARIO COUNTY)

For the Year 1919.

With Reports of Director and Other Officers.

ALBANY
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1920

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STATE OF NEW YORK:

DEPARTMENT OF AGRICULTURE,

ALBANY, *January* 15, 1920.

To the Legislature of the State of New York:

As Commissioner of Agriculture, and as President of the Board of Control, I have the honor to submit herewith the Thirty-eighth Annual Report of the Director of the New York Agricultural Experiment Station, at Geneva, N. Y., in pursuance of the provisions of the Agricultural Law.

I am, respectfully yours,

CHARLES S. WILSON,

Commissioner.

NEW YORK AGRICULTURAL EXPERIMENT STATION.

W. H. JORDAN, *Director.*

GENEVA, N. Y., *January* 15, 1920.

HON. CHARLES S. WILSON, *Commissioner of Agriculture, Albany, N. Y.:*

DEAR SIR: I have the honor to transmit herewith the report of the Director of the New York Agricultural Experiment Station for the year 1919.

Yours respectfully,

W. H. JORDAN,

Director.

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HAROLD J. CONN, Ph.D., *Associate Bacteriologist.*

JOHN W. BRIGHT, M.S.,
[1]GEORGE J. HUCKER, M.A., *Assistant Bacteriologists.*

FRED C. STEWART, M.S., *Botanist.*

WALTER O. GLOYER, M.A., *Associate Botanist.*

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RUDOLPH J. ANDERSON, Ph.D., *Biochemist.*

ARTHUR W. CLARK, B.S.,
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[2]JOHN C. BAKER, Ph.D., *Associate Chemists.*

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WALTER L. KULP, M.S.,
[3]HAROLD L. WINSTON, B.S.,
[4]MILLARD G. MOORE, B.S., *Assistant Chemists.*

GEORGE A. SMITH, *Dairy Expert.*

[5]FRANK H. HALL, B.S., *Vice-Director; Editor and Librarian.*

PERCIVAL J. PARROTT, M.A., *Entomologist.*

HUGH GLASGOW, Ph.D.,
*FRED Z. HARTZELL, M.A. (Fredonia), *Associate Entomologists.*

[6]HAROLD E. HODGKISS, B.S.,
[7]BENTLEY B. FULTON, M.S.,
[8]ROSSITER D. OLMSTEAD, B.S.
[9]CLARENCE R. PHIPPS, B.S., *Assistant Entomologists.*

ULYSSES P. HEDRICK, Sc.D., *Horticulturist.*

[10]R. D. ANTHONY, M.S.A.,
*FRED E. GLADWIN, B.S. (Fredonia),
ORRIN M. TAYLOR,
GEORGE H. HOWE, B.S., *Associate Horticulturists.*

[11]JOSEPH W. WELLINGTON, B.S.
WILLIAM C. STONE, M.S.,
EDWARD H. FRANCIS, M.A.,
[12]THEODORE E. GATY, B.S., *Assistant Horticulturists.*

[13]F. ATWOOD SIRRINE, M.S. (Riverhead), *Special Agent.*

JESSIE A. SPERRY, *Director's Secretary.*

FRANK E. NEWTON,
WILLARD F. PATCHIN,
LENA G. CURTIS,
MAE M. MELVIN,
MAUDE L. HOGAN,
K. LORAINE HORTON, *Clerks and Stenographers.*

ELIZABETH JONES, *Computer and Mailing Clerk.*

Address all correspondence, not to individual members of the staff, but to the NEW YORK AGRICULTURAL EXPERIMENT STATION, GENEVA, N. Y.

The Bulletins published by the Station will be sent free to any farmer applying for them.

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*Connected with Grape Culture Investigations.

[1] Appointed August 12, 1919.
[2] Resigned January 31, 1919.
[3] Appointed April 22, 1919.
[4] Appointed November 16, 1919.
[5] Resigned August 30, 1919.
[6] Resigned April 30, 1919.
[7] Resigned April 30, 1919.
[8] Appointed May 1, 1919.
[9] Appointed July 1, 1919.
[10] Resigned April 30, 1919.
[11] Resigned June 30, 1919.
[12] Appointed July 1, 1919.
[13] Retired July 1, 1919.

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THIRTY-EIGHTH ANNUAL REPORT

OF THE

Board of Control of the New York Agricultural Experiment Station

TREASURER'S REPORT

GENEVA, N. Y., *July* 1, 1919.

To the Board of Control of the New York Agricultural Experiment Station:

As Treasurer of the Board of Control, I respectfully submit the following report for the fiscal year ending June 30, 1919:

1918	RECEIPTS		
July 1.	To balance on hand		$5,430 89
	Salaries, Albany	$79,849 94	
	Labor, Albany	29,529 28	
	Maintenance and operation (last year)	2,087 19	
	Adams and Hatch funds	2,707 07	
	Maintenance and operation	32,942 56	
	Communication	191 52	
	Repairs	2,914 49	
	Demonstration, library, and Administration Bldg	595 00	
	Wages, temporary	50 73	
			150,867 78
	Received for produce sold		3,029 31
			$159,327 98

EXPENDITURES

Salaries, Albany	$79,849 94	
Labor, Albany	29,529 28	
Hatch fund	1,488 19	
Adams fund	1,497 50	
Maintenance and operation (last year)	5,690 05	
Maintenance and operation	27,878 50	
Communication	191 52	
Repairs	2,194 49	
Demonstration, library and Administration Bldg	595 00	
Wages, temporary	50 73	
		$149,685 20
Remitted treasurer State of New York, produce		3,029 31
Remitted treasurer balance appropriations		1,535 10
Balance on hand June 30, 1919		5,078 37
		$159,327 98
Balance Ring Memorial Fund		$1,198 86

All expenditures are supported by vouchers approved by the Auditing Committee of the Board of Control and have been forwarded to the Comptroller of the State of New York.

(*Signed*) W. O'HANLON,
Treasurer.

DIRECTOR'S REPORT FOR 1919.*

To the Honorable Board of Control of the New York Agricultural Experiment Station:

Gentlemen.— It is my duty and privilege to again report to you a year's operations of the institution under your charge, together with a statement of its financial relations and responsibilities and the needs of the institution if it is to efficiently maintain its work.

The Station, like all institutions of an educational character was seriously affected by the war. It can be said, however, that it has now become readjusted to a pre-war basis, and is in a position to go on with its work as efficiently as in the past.

ADMINISTRATION.

STATION STAFF.

An unusual number of changes have occurred during the year in the personnel of the Station Staff. It does not appear that these changes have been due to dissatisfaction on the part of the members of the Staff who have gone to commercial houses or other institutions, but because, owing to the very peculiar and embarrassing policy enforced upon the institution through the present budget system, it is not possible to make such moderate changes in salaries as would retain their services.

As noted in my last report, John C. Baker, Ph.D., accepted a position in New York City, at a salary two and one-half times that he was receiving at this institution. He closed his connection with the Station on Jan. 31, 1919. Dr. Baker exhibited qualities peculiarly fitting him for research work, and the inability of the Station to hold him at the small advance of salary which he was willing to accept is an evidence of the unfortunate effect of our existing budget system.

On April 30, 1919, two members of the Staff accepted positions with the Pennsylvania State College: Roy D. Anthony, M.S., as Experimental Pomologist in the Department of Horticulture; Harold E. Hodgkiss, B.S., as Professor of Extension Entomology.

* **Reprint of Bulletin No. 470, December, 1919.**

Bentley B. Fulton, M.S., resigned his position on April 30, 1919, to accept the position of Assistant in the Department of Entomology of the Oregon Experiment Station.

Joseph W. Wellington, B.S., on June 30, 1919, accepted a position in the Bureau of Plant Industry of the U. S. Department of Agriculture.

The reasons given in all cases for leaving the institution was the necessity for larger compensation. These four resignations have occurred after long periods of service, and it is to be regretted that the institution was not in position to retain experienced men.

It is with great regret that I record the resignation, because of illness, of Frank H. Hall, who for twenty-two years had served the institution as Station Editor and Librarian. Mr. Hall was a pioneer in editorial work performed for an institution of this character. It was his duty not only to edit the publications of the institution but to give a popular expression to the scientific results having a practical bearing that were reached by the Scientific Staff. It is generally conceded that this work was performed with admirable judgment, and the success of the institution and its influence in the agriculture of the State is due in no small part to the services that Mr. Hall rendered. As advisor to the members of the Staff in the preparation of their publications, Mr. Hall proved himself invaluable, and his absence from our number, enforced by physical disability, is a matter for profound regret.

F. A. Sirrine, B.S., who became connected with the institution on July 1, 1902, as Assistant Entomologist, and who later occupied the position of Special Agent of the Station on Long Island, closed his connection with the institution on July 1, 1919. It should be recorded that Mr. Sirrine rendered valuable service to the agricultural people of Long Island, especially in matters pertaining to the control of plant and insect pests. The amount of work which it was possible to do at long distance in that section of the State did not seem to justify the continuance of the office of Special Agent, especially as his time was largely occupied with extension service rather than investigation.

Rossiter D. Olmstead, B.S., was appointed on May first to the position of Assistant Entomologist. Mr. Olmstead is a graduate of Wesleyan University, Middletown, Conn. He served for one year as instructor in Biology in the St. Lawrence University, Canton, N. Y.,

and resigned to enter the aviation service of the U. S. Army, where he served for two years, and received a commission as second lieutenant.

George J. Hucker, M.A., was appointed to the position of Assistant Bacteriologist on August 12, 1919. Mr. Hucker is a graduate of Lenox College, Iowa. He reinforced his under-graduate work with two years' study in Columbia University. He has served as Special Field Agent in the Federal Department of Agriculture, later entering war service where he was appointed to be lieutenant in the Sanitary Corps.

Harold S. Winston, B.S., was appointed to the position of Assistant Chemist on April 1, 1919. Mr. Winston is a graduate of the New York State College of Agriculture. He rendered war service as a member of the Coast Artillery Corps.

Clarence R. Phipps, B.S., a graduate of the Massachusetts Agricultural College spent a year in the aviation service of the U. S. Army. He was appointed to the position of Assistant Entomologist on July 1, 1919.

Theodore E. Gaty, Jr., B.S., is a graduate of the New York State College of Agriculture, and rendered war service for nearly two years as ensign in naval aviation. He was appointed to the position of Assistant Horticulturist on July 1, 1919.

Millard G. Moore, B.S., was appointed to the position of Assistant Chemist on November 16. Mr. Moore is a graduate of the University of Maine, in the Chemical Engineering Course.

George H. Howe, B.S., who entered the institution in 1910, as Assistant Horticulturist, was promoted on April 30, 1919, to the position of Associate Horticulturist.

Miss Laura G. Collison, on September 1, 1919, was appointed to the position of Station Editor and Librarian to fill the position vacated by Frank H. Hall.

MAINTENANCE FUND.

The expenditures of the Station during the fiscal year ending June 30, 1919, were as follows:

Personal service	$107,072 72
Maintenance and operation (including repairs)	60,860 81
Construction or permanent betterments	65,803 99
Total	$233,737 52

The Legislature of 1919 made the following appropriations for the use of the Station during the fiscal year beginning July 1, 1919:

Personal service	
Salaries and wages	$116,650 00
Maintenance and operation	
Fuel, light, power and water	5,000 00
Printing	15,400 00
Equipment and supplies	13,000 00
Hired horses and vehicles	2,500 00
Traveling expenses	3,000 00
Communication	2,200 00
General plant service	750 00
Repairs	2,250 00
Rent	1,000 00
Total	$161,750 00

At a meeting of your Board on September 20, 1919, you adopted a budget for presentation to the Legislature of the present year to cover the expenditures of the institution for the fiscal year beginning July 1, 1920. Subsequent events have led to the recommendation, after mature deliberation, that certain salary items in that budget should be increased above the amounts first recommended. The budget as amended carries the following sums for the various needs of the institution:

Personal service	$148 050 00
Maintenance and operation	52,400 00
New construction and permanent betterments	51,000 00
Total	$251,450 00

Unless the Legislature of the State gives favorable recognition to the requested increases for salaries of the members of the Station Staff, serious results are likely to follow. There appears to be on the part of our national and state legislatures a disposition to give somewhat adequate recognition to the increased cost of living as related to the wages of the industrial class. This increased cost of living affects other classes just as fully, and there is no logic in larger wages for industrial workers, even wages that are above the salaries of teachers or professional workers, without applying the same policy to the employees of the State or Nation.

Certain leading members of the Station Staff have had no increase in salary for nine and a half years, and the argument that they

were either greatly overpaid nine years ago or are greatly underpaid now is unanswerable. It is encouraging that the agricultural people of the State have become aroused to the danger that is threatening their College of Agriculture and Experiment Station through the insufficient payment of the members of the Staffs of these institutions. They have come to recognize that these teachers and investigators will inevitably accept positions at higher salaries, either with similar institutions or in many cases with commercial houses, unless relief is afforded. It appears that committees representing the agricultural people have been formed, the duty of which is vigorously to advocate to the Budget Committee of the State Legislature a liberal increase in the salaries of the members of the faculty of the New York State College of Agriculture and the Staff of the New York Agricultural Experiment Station.

NEW POSITIONS.

Three new positions should be established in the Station Staff, namely, Associate Botanist, Assistant Librarian and Museum Preparator.

Assistant Botanist

The inspection of agricultural seeds is assuming great importance because of adulteration of these seeds and, in many instances, their poor quality. The Experiment Station has been carrying on seed inspection under an inefficient law, and has also examined hundreds of samples of seeds sent in by farmers. A new law, practically the Uniform Seed Law, which is in force in between twenty and thirty states, was passed last winter, but, because of an error, the Governor deemed it wise to veto it in order that it might be passed in satisfactory form by the coming Legislature. Undoubtedly, a new law involving new responsibilities and a larger amount of work will be passed by the Legislature of 1920. It will not be possible to administer this law with the present number in the Botanical Staff, namely, the Chief, his Assistant and a helper, and carry the other work of the Department. The work of an ordinary helper has not been found to be satisfactory as it has needed constant supervision. It is, therefore, believed to be wise to promote the present Assistant Botanist to Associate Botanist and appoint an Assistant Botanist at a salary sufficient to secure reliable service.

Assistant Librarian

The existence of an adequate scientific library properly classified is a primary asset in the equipment of an agricultural experiment station. Moreover, current scientific literature, so far as it relates to the immediate work of the members of the Station Staff, should be properly classified and more or less of it abstracted. Heretofore, the entire care of the library has been placed upon the Station Editor, but the proper editing of the station publications and the writing of the popular bulletins do not leave sufficient time for giving the library the needed attention. It is, therefore, highly desirable that an Assistant Librarian should be appointed, competent to take chief charge of the library under the direction of the Librarian.

Museum Preparator

In the construction of the new administration building at the Experiment Station generous space has been assigned to the placing of a museum, the purpose of which is to visualize the important results which the Station has reached in its investigations. It is greatly desirable that this be accomplished, because many visit the Station for the purpose of gaining some comprehension of what is being done, sometimes along very special lines. The only way in which this knowledge can be imparted in a brief time is by visualizing the results of past investigations. Museum cases are now being constructed, and there should be located at the Station a competent expert who can prepare the exhibits under the eye of the men that are familiar with the results accomplished.

The work in Horticulture and in the control of various pests offers a fine opportunity for a museum of the character indicated. It is not possible for the members of the Station Staff to prepare these museum materials, which is a time-consuming piece of work, and at the same time carry on the work under their charge.

THE BUDGET SYSTEM AS RELATED TO EDUCATION AND RESEARCH.

Some time since, your Director assumed the responsibility of issuing a pamphlet discussing the relation of the budget system to public welfare, especially to education and research in this State. While much has been said and written on this topic, its importance is such as to justify a continued appeal for changes that are essential to the welfare of at least some of the State institutions. The following is a quotation from the pamphlet mentioned:

"A budget, whether national or state, should reflect public needs, and only public needs. This requires that the information upon which departmental and institutional appropriations are based should be obtained from administrative officials who understand in detail what is demanded and upon whom will rest the responsibility of handling the funds. The formulation of a budget from the point of view of decreasing or increasing former appropriations may be utterly irrational. The advice of the accountant may often wisely be disregarded.

"The budget should be sufficiently flexible in its provisions to meet the demands of the various state departments and institutions, or else be differentiated to meet the requirements of dissimilar state agencies having an utterly unlike function. To apply the same fiscal system in all its details to a prison and to a university or college may be absurd, in fact, as would be the practice of prescribing the same medical treatment for typhoid fever and pneumonia.

"A budget should be sufficiently flexible to meet unforeseen demands without undue embarrassment. In view of the frequent changes in the requirements for personal service and in the cost of supplies and other maintenance items, an inflexible budget, closely segregated for specific purposes, may become a serious administrative handicap. This is fully realized by officials who have had to deal with such a situation.

"The fiscal system should give full play to the judgment of administrative officials. To seriously limit administrative autonomy is to render officials irresponsible. More than this, such limitation suppresses rather than encourages the initiative, only through the exercise of which public service may be improved.

"A close analysis of the provisions and limitations of the budgetary system now in force in the State of New York shows that, with a single exception, it lamentably fails to meet these specifications. The method by which the financial committees of our legislature secure the information upon which to base their budgetary recommendations is the one feature that is above serious criticism. The officials responsible for the management of state departments and institutions are given full and extended hearings on the recommendations which they have previously presented at the request of these committees, at which times the items of the budget proposals are discussed in great detail. The administrative point of view is

given the most courteous and careful consideration, but it remains true, nevertheless, that the final determination of salaries and the segregation of the total amount of money appropriated into items for specific purposes rests with the legislative committees. Heads of state departments and institutions realize that these committees do not always adequately recognize in their conclusions special conditions and needs which can only be understood through intimate experience.

"The budget of the State of New York, so far as it applies to departmental and institutional activities, is formulated in flexible terms. The salary for appointive positions, with the exception of the wages paid common laborers, is fixed, and may not be exceeded. No salary contingent fund is provided, and a transfer may not be made from one salary item to another. This proves to be a most serious handicap, especially in the management of research and educational institutions. These institutions must meet competition from other states in the appointment and retention of members of their staffs. It is peculiarly true of the State College of Agriculture and the Experiment Stations that this competition is keen. With fixed salaries, administrative heads are helpless to defend their institutions against the inroads of similar institutions and the inducements offered by commercial interests, and they are at the present time losing men who sometimes could be profitably retained by a readjustment of salary expenditures without increasing the total salary appropriation. The chief function of a Dean of a College of Agriculture or the Director of an Experiment Station is the selection and retention of his associates, and, under the conditions prevailing in the State of New York, his autonomy in the exercise of this function is often disastrously limited. The result of this is already seen in a lowering of the standard of service in agricultural education and research in New York. If the present budgetary policy as applied to personal service is allowed to continue, efficiency in scientific investigation and in education, so far as these efforts are supported by public funds, will almost certainly fall below the standards maintained by privately endowed institutions.

"This inflexible budget is applied to all state agencies alike, ignoring their greatly varying functions and the great differences in the extent to which their operations may be standardized. Institutions whose problems and expenditures may not be intelligently

scheduled months in advance are hedged about by the same fiscal regulations as departments whose work is clearly clerical and the activities of which may be quite definitely stabilized.

"The funds provided for the maintenance and operation of our State departments and institutions is segregated in inflexible terms under something like sixteen heads. These divisions include "Fuel, Light, Power and Water," "Printing," "Equipment," "Supplies," "Traveling Expenses," "Communication," "General Plant Service," "Repairs" and so on. The price of coal may materially change; railroad fares and hotel expenses may be greatly increased; higher freight and express rates may be established; postage may be changed from two cents to three cents; the wages of industrial labor involved in making certain repairs may be materially raised, and yet upon administrative officials is placed the perplexing and often exasperating burden of carrying on the activities under their charge with a fixed, highly segregated budget, adopted months in advance of the current fiscal year, which takes no account of all these variations in expense.

"The question may be asked,— Do not these officials recommend the items of the budget? They certainly do, but their recommendations, based upon past experience, which is a treacherous guide, are called for on the first of October for a fiscal year beginning on the first of next July, and no man has the prophetic vision which enables him to foresee the maintenance expenses which may be necessary in the way of repairs, some of which are accidental in their origin, or the industrial changes which greatly increase the cost of operating institutions of all kinds. It is rational to ask for these detailed recommendations, but it is irrational to adopt them in an inflexible form. It is a serious question whether a careful analysis of past and present expenditures would not show the present fiscal plan to be wasteful rather than economical.

"If, then, the budgetary system now in force in New York is unsatisfactory, what method of financing the State can be adopted that will accomplish the desired control of public funds? Without question, the people have a right to detailed information as to how their contributions to the State Treasury are expended. It cannot reasonably be denied that the budgetary policy now in operation has some commendable features. The present method of obtaining information about needed appropriations and the submission of

budget proposals in detail are essential features of an efficient fiscal system. The figures so obtained should be co-ordinated and adjusted to the financial resources of the State and then published in available form for legislative use and public enlightenment.

"But if a reasonable autonomy of administrative officials is to be maintained, if the boards and other officials of the State are to be in certain particulars something more than rubber stamps, if a flexibility is to be given to the use of funds that will permit responsible officials to exercise their judgment under unforeseen and varying conditions, if real needs rather than the confines of a rigid fiscal structure are to determine expenditures — then legislative appropriations should be made in lump sums or in broadly segregated items. Judgment as to the proper and legitimate use of funds should then be based upon a comparison of the detailed budget proposals with the actual expenditures. An explanation might well be demanded concerning a wide departure from the departmental and institutional requests. Probably the addition of a liberal contingent fund provision or the permission after adequate explanation to transfer from one budget item to another would render the present plan much more workable. Under either method, with proper accounting and severe auditing, the people of the State might feel assurance that the expenditure of their money is sufficiently safeguarded."

MAINTENANCE OF RESEARCH.

It is very evident that agricultural research should have every possible encouragement at the present time. For two reasons, at least, there is real occasion for solicitude concerning the future maintenance of Experiment Station activities. In the first place, the Stations staffs are being depleted of some of their most useful members by the very attractive salaries offered by commercial enterprises. In the second place, Station activities are more or less submerged by the rapid development of the extension service. In 1917–18, the funds available for extension work from federal and state appropriations was almost twelve millions of dollars; in 1918–19 over sixteen millions of dollars. In 1917, the federal and state funds available for the Stations amounted approximately to three million, nine hundred thousand dollars. It appears that the financial support given to the popular extension of knowledge is at least three times larger than that given to its production.

Because of this generous support, the extension movement has made very rapid growth, and, as a consequence, has successfully invaded Experiment Station staffs in order to secure the necessary service. More important than this is the fact that young men, with little post graduate experience or training, desirous of entering the agricultural field, have found it possible to secure at once in extension work salaries equal to those paid for the higher grade of scholarship and experience necessary to successful research, thus avoiding the use of time and the expense involved in more extensive preparation for the service in Experiment Stations which they otherwise might have entered.

It is obvious that research supplies the subject matter used for teaching in the class room and in the field, and there are many unsolved problems yet untouched, and others that have not been invaded beyond the outskirts, concerning which the teacher must as yet be silent. It surely is irrational to spend millions in agricultural extension, and reduce to a mere pittance the sum applied to the acquisition of the fundamental knowledge.

PUBLICATIONS.

It is gratifying to know that the Legislature of 1919 made appropriation for the publication of "The Pears of New York" and "Sturtevant's Edible Plants." The latter is now passing through the press. It is the work of a distinguished botanist, the first Director of this Station. It is unique in character, and probably contains the most comprehensive information available concerning plants which may be used as food for the human family of any work issued in this country or elsewhere. "The Pears of New York" is nearing completion, and the manuscript will probably be submitted to the State Printer within the present calendar year.

The number of names to which the Station publications are now issued in this State and others is as follows:

POPULAR BULLETINS.

Residents of New York	37,500
Residents of other States	2,497
Newspapers	744
Experiment Stations and their staffs	2,400
Miscellaneous	350
Total	43,491

COMPLETE BULLETINS.

Experiment Stations and their staffs	2,400
Libraries, scientists, etc	400
Foreign list	432
Individuals	4,154
Miscellaneous	350
Total	7,736

BUILDING NEEDS.

Buildings erected at State institutions are not immune to decay. It seems almost futile to call attention to the fact that the forcing houses of this institution were erected somewhere about thirty years ago, and are now in such condition that a disaster resulting in the loss of the contents of the houses may occur at any time. It is difficult to understand why there is any reluctance in replacing buildings of this character when they are needed for important work. If the State is to continue in its efforts to improve the fruits of the State, and exhibit the results of its work at the State Fair and at the meetings of the Horticultural Societies, the cold storage plant which is small and now considerably decayed must be replaced by a more efficient structure. The budget for this year includes an item covering the probable cost of these two buildings.

RESULTS OF STATION WORK IN 1919.

There is presented here only a brief summary of the results accomplished. These are conveyed to the constituency of the State through the use of bulletins, any of which may be obtained on application by residents of the State. It is proposed to issue, some time during the year 1920, a comprehensive summary written in a more or less popular form of the important results which the Experiment Station has reached that have a direct bearing on agricultural practice and conditions.

BACTERIOLOGICAL AND DAIRY DIVISIONS.

Ammonification of manure in soil.— The results of this work have been published as Technical Bulletin No. 67. This report primarily discusses the part that two common soil organisms (*Pseudomonas fluorescens* (Flügge) Migula and *Ps. caudatus* (Wright) Conn play in the ammonification of manure after this is added to soil. The proof seems to be complete that these bacteria are active ammonifiers

under the conditions specified. Yet it seems probable that other organisms may take equally as active a part in the process, and that other bacteria take the lead in the process when it takes place in the manure pile before addition to soil. *Bacillus cereus* Frankland, an organism commonly believed to be active in ammonification processes, was not found to be active under the conditions tested.

Further studies of the organisms concerned in the ammonification of manure, and of the effect on the flora of adding phosphates, peat, straw, and like materials to manure have been made during the year, but no report on the results has been prepared as yet. A part of this work has been done in coöperation with the Division of Agronomy.

Taxonomic work.— As bacteriologists find great difficulty in distinguishing between the types or species of bacteria concerned in specific agricultural processes, some time has been given during the year to a study of methods useful for this purpose. In fact, more attention has been given to this subject than usual in order to aid in the preparation of a general committee report on "Methods of pure culture study" drawn up for the Society of American Bacteriologists under the chairmanship of a member of the department. Some of these studies have been discussed in Technical Bulletin No. 73 under the title "The use of the nitrate-reduction test in characterizing bacteria."

Two papers entitled "Comments on the classification and evolution of bacteria" and "The nomenclature of the Actinomycetaceae" have been published from the department in the Journal of Bacteriology. These were written as the results of discussions started thru the formulation of a general classification of bacteria by a committee of bacteriologists. As all research in agricultural bacteriology is being badly retarded by the lack of a generally approved classification of bacterial species, the committee report promises to be generally useful in our work.

Physiological and fermentation studies of milk.— Two pieces of investigation have been completed during the year in coöperation with the Division of Chemistry. The first of these is a physiological study, and deals with the reaction of fresh milk as influenced by the entrance of blood serum during secretion. A report of the work is in process of preparation. The report of the second investigation has been completed, and is being published as Technical Bulletin

No. 74 under the title "Relation between lactic acid production and bacterial growth in the souring of milk."

Methods of counting bacteria in milk.— A report on the series of milk analyses carried out in coöperation with the Department of Dairy Industry of the College of Agriculture at Ithaca has been completed, and is to be published as Technical Bulletin No. 75. The results secured, together with those previously presented (Bulletins Nos. 439 and 443), show without question that current conceptions regarding the number of bacteria in milk must undergo radical modification. The numbers of bacteria present are greatly in excess of the numbers usually given as the result of analyses made by the agar plate method. The latter figures really show the number of groups of bacteria capable of developing on agar under the conditions maintained. As the groups of bacteria in fresh milk commonly contain an average of from two to six or eight bacteria, and frequently contain more than these numbers, the figures ordinarily given should be corrected by multiplying by the number of bacteria per group. As the errors introduced by the clumping of the bacteria are so large and so irregular, it is evident that the doubts that many bacteriologists have had regarding the accuracy of these counts have been fully justified.

While these analyses show that it is possible to make reasonably accurate estimates of the number of individual bacteria present where conditions are favorable for accurate work, yet the fact that these conditions are uncommon makes accurate work impossible in most cases. However, the results secured in the grading of milk by routine methods into two or three classes according to the number of bacteria present, as previously reported in Bulletin No. 443, show that the methods now in general use for this purpose may be made to yield results of sufficient accuracy to justify this use. It is unfortunate that dairy analysts still continue the custom of reporting agar plate counts as if they indicated the number of individual bacteria present. This custom gives the layman an erroneous idea of the accuracy of the counts.

Dairy sanitation and tuberculosis studies.— During the year, attention has been given to the testing, under practical farm conditions, of the methods of cleaning milking machines developed at the Station. A report on this work is in process of preparation. The department has also coöperated with the Extension Service of the

College of Agriculture in adapting these methods to the needs of the dairy farmers of the State.

The past year has been the fourteenth since a case of demonstrated tuberculosis has occurred in the Station herd. During this time the tuberculin test has been made regularly, and three apparent reactions have been found; but careful autopsy has failed to reveal any sign of tubercular lesions. A report, giving the facts in detail, has been made during the year to the New York State Dairymen's Association, and this will appear in the proceedings of the association. It is felt that this good record has been secured thru the development of the herd without the introduction of any outside stock other than pure bred sires. The latter have been brought from herds known to be free from tuberculosis. The herd at the present time is composed of pure bred or practically pure bred Jerseys. During the past five years the amount of milk produced per cow has varied between 6,204 and 7,251 pounds. This milk has shown an average butter fat percentage of about 5.8.

TESTING OF BABCOCK GLASSWARE FROM DECEMBER 1, 1918 TO DECEMBER 1, 1919.

Ten percent milk bottles	15,042
Eight percent milk bottles	16,626
Thirty percent 9-inch, 18-gram cream	1,044
Thirty percent 6-inch, 9-gram cream	126
Thirty percent 6-inch, 18-gram cream	655
Forty percent 6-inch, 18-gram cream	242
Fifty percent 6-inch, 18-gram cream	1,372
Fifty percent 6-inch, 9-gram cream	1,631
Fifty percent 9-inch, 9-gram cream	238
Fifty percent 9-inch, 18-gram cream	138
17.6 pipettes	4,113
18 c.c. pipettes	109
9 c.c. pipettes	186
10 c.c. pipettes	93
8.8 c.c. pipettes	13
Skim-milk bottles	192
Acid measures	446
Total	42,266
Rejections	362
Express packages sent out	749

Coöperative Studies — During the year the Station has continued to coöperate with the city of Geneva in directing the milk inspection work for the city. This has given many opportunities to test out laboratory findings under field conditions. The work has

yielded results of value in connection with the milking machine investigations, investigations on the relation between the cooling of milk and its bacterial quality, prevalence of garget, effect of utensils on the bacterial contamination of fresh milk, and the like. During the coming year it is expected that further summaries of the entire series of stable and milk sanitation studies carried out at this and at the Illinois Station will be prepared, so as to make the findings of more direct use to the dairymen of the State in their efforts to produce milk containing few bacteria.

BOTANICAL DIVISION.

Missing hills in potato fields.— Among potato growers there is much difference of opinion concerning the effect of missing hills upon the yield. Some assume that they are a total loss, while others hold that a large part of the loss is made up by the increased yield of adjoining plants. Even among professional experimenters there is lack of agreement as to the proper method of comparing the yields of plats differing in percentage of stand.

An experiment conducted by the Station during the season of 1918 was designed to shed light on this point.

At planting time, each of 360 potato tubers was divided lengthwise into two equal pieces. The 720 seed-pieces so obtained were planted 15 inches apart in the row in groups of four with blank spaces of 30 inches between groups. In other words, every fifth hill was a missing hill. Each group of four contained the two pairs of seed-pieces from two tubers. Hence, one member of each pair of the resultant plants adjoined a missing hill. The difference between the yield of this member (the exterior plant) and the yield of its mate (the interior plant) was taken as the measure of the influence of the missing hill.

Data were obtained from 351 pairs of plants. In weight of total yield the exterior plants outyielded the interior ones by 23.2 percent on the average. Accordingly, the answer given by the experiment is that, under such conditions as obtained in this experiment, the loss from missing hills is offset to a considerable extent by the increased yield of adjoining plants. In the case of a skip consisting of a single missing hill the two adjoining plants (one on either side) together make up 46.4 percent of the loss in total yield, and a little more in yield of marketable tubers.

As a sort of check on the experiment, an attempt was made to determine the magnitude of the variation in yield between the two members of a pair of plants from the same tuber when grown under conditions as nearly parallel as they could be made. For this purpose pairs of seed-pieces similar to those used in the experiment proper were planted in continuous rows without blank spaces. Data were obtained from 85 pairs of plants. In different pairs the difference in total yield varied from 0 to 66.7 percent of the mean yield of the two plants of the pair, the average being 20.7 percent. Such a wide variation in yield between plants under supposedly parallel conditions indicates that there are factors having a very important bearing on the yield of potatoes which are either unknown or not estimated at their proper value.

The details of this experiment have been published in Bulletin No. 459.

Seeds and seed testing.— The work done along this line during 1918 has been reported in Bulletin 462. In addition to tests of 179 official seed samples collected by agents of the Commissioner of Agriculture, and analyzed for purity in accordance with the provisions of the State seed law, there were made 287 purity tests and 396 viability tests of unofficial samples sent in by farmers and seed dealers. A study of the results of these tests shows plainly the need of a stricter law governing the sale of agricultural seeds. It should be required that every lot of seed offered for sale in the State shall bear a label showing its approximate purity and viability.

The seed mixtures upon the market, particularly the cheap mixtures offered by mail-order houses, are notorious for impurity. They should be avoided. When mixtures are desired, the different kinds of seed required should be purchased separately, and mixed at home.

In the spring of 1918 the viability of seed corn was very generally quite low. Under such conditions it is highly important that all seed corn used should be tested for viability. For this purpose the home-made rag-doll seed-tester is very satisfactory. Often much time and labor may be saved by rejecting, without test, all ears having kernels with amber-colored germs because they are likely to have been injured by freezing.

Miscellaneous notes on plant diseases.— Incidental to his principal line of research, the Station Botanist has opportunity for making

observations upon a great variety of plant diseases. Some of these, altho fragmentary, are worthy of record, and so it has been deemed advisable to occasionally publish collections of them in bulletin form. Bulletin No. 463, published in June 1919, contains about 40 such notes, the most important being the following: A stem-and-root disease of apple and pear seedlings, the cause of which has not been determined; a bacterial soft rot of the fleshy petioles of the spotted arum; cabbage blackleg, an important fungus disease which is becoming rather common in the State; black leaf-speck of cabbage, cause undetermined; a destructive storage rot of carrots caused by a fungus and a bacterium; an insect twig blight and leaf spot of catalpa resembling a fungus or bacterial disease; a destructive anthracnose of red clover caused by the fungus *Gloeosporium caulivorum;* crinkle leaf, a non-parasitic disease of currants; an instance in which a severe attack of *Fomes ribis* on currant bushes appeared to do them no harm; *Hypholoma perplexum* growing upon living canes of red currant; gooseberry powdery mildew attacking leaves and twigs of red and black currant; a white, crystallin deposit on currant canes; a witch's broom of unknown origin on red currant; and an injury to the trunk of an elm tree caused by enclosing it in a box "protector."

Control of dandelions in lawns.— Experiments made at the Station during the past eight years show that dandelions may be eradicated from lawns by proper spraying with a solution of iron sulfate. The treatment is comparatively inexpensive, and does not materially injure the grass. Usually, four or five applications are required. The first spraying should be made in May just before the first blooming period of the dandelions. One or two others should follow at intervals of three or four weeks; and, finally, one or two more in late summer or fall. During the hot, dry weather of midsummer the spraying should be discontinued because of the danger of injury to the grass. A conspicuous blackening of the lawn which follows each spraying soon disappears if the grass is growing vigorously. Of the other common lawn weeds some are killed while others are but slightly injured by spraying. Unfortunately, white clover, also, is killed.

Tests of certain methods of supplementary treatment, such as reseeding, liming of the soil, and the use of commercial fertilizers and stable manure, were made in conjunction with the spraying

experiments. The results obtained warrant the strong recommendation that spraying be supplemented by the use of fertilizers and the application of grass seed in the spring and fall of each year. With proper management a lawn may be kept practically free from dandelions by spraying every third year.

The cutting-out method of fighting dandelions is laborious and ineffective unless the greater part of the root is removed. Shallow cutting, unless done frequently, is worse than none at all, because each cut-off root promptly sends up one or more new plants.

A full account of these experiments has been published in Bulletin No. 466.

FIELD EXPERIMENTAL WORK OF BOTANICAL DIVISION.

Potato experiment........................L. L. Foote, Malone.
F. A. Sirrine, Riverhead.

DIVISION OF CHEMISTRY.

BULLETINS PREPARED IN 1919 BY CHEMICAL DEPARTMENT.

1. Analyses of 588 samples of commercial fertilizers.
2. Analyses of 1607 samples of feeding stuffs.
3. Carbonic acid and carbonates in cow's milk.
4. Conditions causing variation in the reaction of freshly-drawn milk.
5. A method for the preliminary detection of abnormal milks.
6. A method for the determination of the keeping quality of milk.

The amount of CO_2 present in cow's milk in the udder is, under normal conditions, about 10 percent by volume. It may fall as low as 7 percent and, in case of diseased udders, may rise to above 80 percent. The CO_2 is present as one part of H_2CO_3 and two parts of bicarbonate.

In studying the variation of reaction in milk, the results are expressed in terms of hydrogen ion concentration in the form of values of pH. In examining some 300 samples of freshly-drawn milk, the pH value is found to vary from 6.50 to 7.20. It varies with the composition of the milk. In general, with a decrease of acidity (as indicated by an increase of pH value) there is a marked tendency toward a decrease in specific gravity and in percentage of fat, solids-not-fat, casein, and lactose, but an increase in proteins other than casein, and in ash and chlorine. These changes in composition are such as would be expected in case blood-serum or lymph were added to normal fresh milk. Diseased conditions in the udder may cause such addition.

A method is much needed in official milk inspection to enable the inspector to select suspicious samples quickly, and then take samples of such milks for further detailed examination in the laboratory. Such a method has been worked out here, one which depends upon the effect of milk upon a solution of a dye called brom-cresol purple. One drop of this solution is added to 3 cc. of milk, and the color is observed. Normal fresh milk gives a grayish-blue color. The production of a lighter or darker color serves to awaken suspicion in regard to the normal character of the milk. The color is made lighter by acids, acid salts, formaldehyde, and also by heating above the usual point of pasteurization. The color becomes deeper blue in the case of milk from diseased udders, watered milk, skimmed milk, and milk containing added alkaline salts. In the inspection of milk, a sample is taken for further detailed examination in the laboratory, if the color is sufficiently lighter or darker than normal to indicate the probability of some abnormal condition. The method has been applied to about 600 samples of market milk with satisfactory results.

There has been no recognized satisfactory test for determining the keeping quality of milk, by which is meant the length of time milk remains sweet, and otherwise palatable and suitable for direct consumption. A method has been devised here for making such a determination, using the same solution of brom-cresol purple that is used in detecting abnormal milks. The same precedure is followed, except that the pipettes and test-tubes used are sterilized before sampling the milk, and, further, the samples of milk in the test-tubes must be incubated a given time at a definite temperature. The milk is examined for changes of color after certain intervals. The principal factor shown by this test as related to keeping quality is production of acid, but additional factors to be observed in connection with it are coagulation of casein, digestion of casein, production of alkali, production of gas, development of abnormal odor, or taste.

DIVISION OF ENTOMOLOGY.

Control of Green Apple Aphis in Bearing Orchards.

The green apple aphis is a common dwarfing and deformative agent of the new growth of younger trees. It prefers succulent tissues, such as exist on terminal growths and water sprouts, and is generally present in injurious numbers for more or less extended

periods during the summer months in nursery plantings and young apple orchards. In occasional years it is very abundant and destructive in old apple plantings. The dwarfing of fruit and defoliation of affected branches have established its status as an important apple pest. The results of this study as presented in Bulletin No. 461 show that oviposition by *Aphis pomi* occurs in the autumn, and the eggs hatch the following spring. The maximum numbers of newly-hatched nymphs are ordinarily observed as color is showing in the leaf tips of the opening blossom buds. Development of the insects is rapid, winged forms of the second generation appearing during late May or early June, when there is a migration to other plantings. The species breeds continuously thruout the summer, producing many broods, which vary in size and number according to seasonal conditions. The invasion of fruit clusters may be attended with dwarfed, misshapen apples which display pimpling and red stippling of the surfaces.

In the Station experiments, the delayed dormant treatment protected bearing orchards until about the middle of June, when there was a reinfestation from winged migrants. While plantings that were given the delayed dormant treatment were not conspicuously injured by the aphis, the experiments so far conducted do not indicate conclusively that this treatment may safely be relied on to afford reasonable and satisfactory commercial control. A spraying during midsummer resulted in the efficient control of the green aphis. Following the treatment there was noticeable improvement in the conditions of apples in most orchards with respect to shape, size and freedom from reddish discolorations.

Comparative tests of nicotine sulphate with soap or large amounts of lime indicated few differences in insecticidal qualities of these preparations. The advantages of the lime wash were the deterrent action on the aphids, and its cleansing properties to the fruits. On account of its lack of surface tension and the difficulty and cost of application to large trees, the use of the lime mixture should properly be limited to young, non-bearing trees or those of moderate size. The rapid killing with nicotine sulphate in combination with soap and its greater spreading properties point to its superiority for large trees. It is probable, for these considerations, that apple growers having trees of great height with widespread branches will continue

to place their dependence on the nicotine-sulphate-soap spray for the control of the green aphis.

The conclusions drawn from the Station experiments are that, in regions where aphids are annually destructive or attacks are apprehended, reliance should be placed on the delayed dormant treatment with lime-sulphur and nicotine sulphate, and on a supplementary spraying during midsummer with nicotine sulphate and soap when the green aphis threatens to develop to destructive numbers on fruit clusters.

The Rosy Aphis in Relation to Abnormal Apple Structures.

Of the various species of aphids that exist on the apple tree, the rosy aphis is conspicuous for its partiality for the foliage of blossom and fruit clusters and, by reason of this preference, the presence of large numbers of the insects on the host is generally attended with damage to both foliage and fruit. Technical Bulletin No. 66 presents with considerable detail various effects of the insect on the size, color and internal structures of apple fruits. Of special interest to growers is the fact that apples attacked by the rosy aphis usually display suppression of the transverse and axial diameters. Inhibition of growth occurs to a greater extent with the transverse diameter. The injury varies in extent, even with fruits of the same cluster, and the amount of damage is largely determined by the earliness and intensity of attacks, and the duration of the period of infestation. The shrinkage in yield on account of the so-called aphis apples is obvious. There should also be taken into account another source of loss — that many apples from infested trees, while marketable, may be below normal size. Besides being smaller, affected apples are frequently poorly colored, and are often not symmetrical in shape. Aphis apples usually have, on an average, fewer seeds than normal fruits. Severely malformed fruits sustained no reduction in the number of primary fibro-vascular structures.

Comparison of Methods for Computing Daily Mean Temperatures: Effect of Discrepancies upon Investigations of Climatologists and Biologists.

The daily mean temperature is usually determined by taking the average of the highest and lowest temperatures that occur during twenty-four hours, readings being taken at a fixed hour of observation

and from accurate maximum and minimum thermometers. The hour of observation is usually in the evening, but each observer uses a convenient hour, the most common time being from 5 P. M. to 8 P. M. The true daily mean is given by a summation of the average hourly temperatures, divided by twenty-four. Comparison of the daily means calculated by the two methods shows that, thruout a year, important discrepancies occur and that these discrepancies are the greater the longer the period from the hour of observation to the following midnight. By means of statistical analysis the effect of the daily discrepancies upon the daily mean, monthly mean, and annual mean temperatures have been determined. The deductions are so numerous that the reader is referred to Technical Bulletin No. 68, which contains the results of this study.

Since biological activity is rather closely related to thermal influence, it is important that the temperature data be exact, otherwise important relationships may be masked. It was found that the approximate means gave very misleading data when daily differences are considered, and that thermograph averages alone can be depended upon in the scientific investigation of the effect of heat upon plants and animals. These considerations are of importance in some phases of agricultural research.

The activities during the summer at Fredonia were directed to two principal phases: the investigation of the life history and control of the Red-headed Systena (*Systena frontalis*), and the testing of new forms of arsenicals on grape foliage. The Red-headed Systena was abundant during the past season and, as it annually injures the young vines (cuttings) in the nurseries as well as doing much damage to young and even old grape vines, it was deemed of sufficient importance to study. The life history had not been investigated to any extent before this season.

Of late years a number of new arsenical poisons have been placed upon the market, and, as a number of these are considerably cheaper than the standard arsenate of lead, it was decided to try all that could be obtained on grape foliage. The result has been that all were found injurious to Concord grape foliage, so the growers are very strongly advised to use the standard materials for spraying.

Since the grape root-worm is becoming more abundant, constant observations were made for vineyards in which the pest might be

numerous, both with the aim of conducting further investigations, and to keep the growers informed of any threatened outbreak. At the same time notes were kept of the activities of other grape pests, and insectary investigations of life histories were continued.

During the season much attention was given to the study of ecology with special reference to grape pests. The object of this investigation was to determine the conditions most favorable for such insects, and to compare these with vineyard conditions to determine the possibility of certain insects becoming vineyard pests. At present writing it seems that the reason certain insects which attack vines of all species under natural conditions cannot thrive under vineyard conditions is that at some critical stage of their life they are unable to live because either moisture or temperature conditions make this impossible, the difference in the two habitats being greater than their adaptability in this respect. Another factor is that the undisturbed conditions in a native habitat permit them to pass thru certain stages, while in vineyards the cultivation of the soil and other operations destroy them.

COÖPERATIVE EXPERIMENTS.

Nature of activity.	Coöperator.	Location.
Control of sinuate pear-borer	Dewitt C. Haight	Croton Falls.
Control of pear thrips	Webster Coons	Germantown.
Control of pear thrips	V. E. Litchenhan	Germantown.
Control of pear thrips	A. W. Hover	Germantown.
Control of pear thrips	Wessel Ten Broeck	Hudson.
Control of pear thrips	F. B. Harrington	Hudson.
Control of pear thrips	Fred and William Hallenbeck	Hudson.
Control of cabbage aphis	McKay Brothers	Geneva.
Control of pear psylla	Middlewood Farms	Varick.
Control of pear psylla	Fred Hammond	Geneva.
Control of pear psylla	McKay Brothers	Geneva.
Control of rosy aphis	W. H. Powell & Son	Lewiston.
Control of rosy aphis	S. F. Burton	Ransomville.
Control of rosy aphis	G. E. Manning & Son	Ransomville.
Control of rosy aphis	A. F. Dale	Lockport.
Control of rosy aphis	F. M. Bradley	Barker.
Control of rosy aphis	G. E. Mead	Barker.
Control of rosy aphis	W. S. Silsby	Gasport.
Control of rosy aphis	H. H. Freeman	Kent.
Control of rosy aphis	John Beckwith	Lyndonville.
Control of rosy aphis	G. E. Snyder	Albion.
Control of rosy aphis	C. M. Harding	Knowlesville.
Control of rosy aphis	H. E. Wellman	Kendall.
Control of rosy aphis	John Beckwith	Demster.
Control of rosy aphis	Maxwell Brothers	Geneva.
Control of cherry aphis	McKay Brothers	Geneva.

HORTICULTURAL DIVISION.

A test of commercial fertilizers for grapes.— In the spring of 1914 this Station published in Bulletin No. 381 the results of a five-year test of commercial fertilizers on grapes. Another five-year period has elapsed, and Bulletin No. 458 is published to show the results of fertilizers on grapes for the added period. The work was carried on by Fred E. Gladwin in the experimental vineyard at Fredonia, N. Y.

The tests show that nitrogen, phosphorus, and potassium have had a marked beneficial effect upon wood growth, yield, and quality of fruit. The data indicate that, of the three elements, nitrogen has been most helpful.

Potassium has given more pronounced results than phosphorus up to the present, altho the latter has had a more beneficial effect upon the green-manure crops in the vineyard.

Nitrogen has not only favorably affected the growth of wood, but it has increased the fruit, and given larger berries and clusters. Phosphorus and potassium have increased the production of wood and fruit, but have not influenced the quality of the fruit to the same extent as the nitrogen. Potassium has caused earlier ripening of the foliage than the other elements.

Even tho the same number of canes be tied up for fruiting purposes, the data show that the fertilizer plats have produced a decided gain of fruit over the unfertilized.

The foliage, after the first few years, has been of better color and size in the plats to which nitrogen was applied. That from the phosphorus and potassium plats ranked second, with that from the check a poor third.

Twenty years of fertilizers in an apple orchard.— In Bulletin No. 339 of this Station, published in 1911, the results are presented for the first seven harvests in a fertilizer experiment which was begun with the planting of the trees on the Station grounds in 1896. The present bulletin discusses the results secured in eight additional harvests.

The factors considered in interpreting results are yield and size of fruit and tree growth. In nearly all cases the result given in the tables is the average of the five trees in the plat. The results may be summarized as follows:

Adding acid phosphate at the rate of 340 pounds per acre per year has not given a noticeable increase in yield.

The addition of 196 pounds of muriate of potash to the 340 pounds of acid phosphate seems to have resulted in an increased yield.

The annual application of 50 pounds of readily available nitrogen in addition to the phosphoric acid and potash has caused no increase in yield.

Plats receiving stable manure have yielded no more than the check plats.

In general there are so many inconclusive or contradictory results that no conclusion of practical value can be drawn from the yields.

When we compare the rank in yield of the plats for the period ending in 1910 with the rank for the last eight years we see a tendency of the checks and phosphoric acid plats to take a slightly lower rank as the experiment has continued.

The average percentage of fruit grading two and one-half inches or larger is given for each plat. There is a greater difference between two nearby check plats than between any fertilized plat and its nearest check. This, together with the variations among the duplicates of the fertilizer treatments, makes it impossible to draw any definite conclusion as to the effect of treatment upon size of fruit.

The average trunk diameter and the approximate average tree volume for each plat are excellent factors to use in comparing the various plats. The two phosphorus and potassium plats lead their adjoining plats both in size of trunk and in tree volume, but it is not possible to say whether the increases are due to the potassium or the combination of the two elements, or to some tree or field variation which does not show.

Heavy applications of nitrogen in a complete fertilizer and in manure have not increased tree growth.

When the costs are considered, certain plats have given increases sufficient to equal the costs, or even to show a profit, but in other plats the same plant food elements have shown a financial loss.

If the results continue in the present direction for another ten years, the increased yields may justify the recommendation of one or two of the treatments, but at present this cannot be done.

These results are from a cultivated orchard on soil naturally well supplied with the plant food elements. On thin, infertile soils or in sod orchards, the results might be quite different.

PUBLICATIONS ISSUED DURING 1918.

BULLETINS.

No. 446. January. Seed tests made at the Station during 1916–1917. Parts I and II. M. T. Munn. Pages 53. Distributed May 15, 1919.

No. 447. February. Newer varieties of strawberries. O. M. Taylor. Pages 23, plates 9. Distributed May 15, 1919.
Popular edition, pages 10. Distributed October 30, 1919.

No. 448. February. The velvet-stemmed Collybia — a wild winter mushroom. F. C. Stewart. Pages 19, plates 11. Distributed June 1, 1919.

No. 449. March. A non-parasitic malady of the vine. F. E. Gladwin. Pages 15, plates 3. Distributed May 15, 1919.

No. 450. July. Control of bacteria in milking machines. Parts III and IV. Pages 68, plates 2. Distributed June 12, 1919.
Popular edition, pages 15. Distributed October 30, 1919.

No. 451. September. Leaf-hoppers injurious to apple trees. Pages 15, plates 4. Distributed June 12, 1919.

No. 452. December. Commercial fertilizers. Pages 253. Distributed May 28, 1919.

No. 453. December. Control of grape-root worm. Pages 332. Distributed May 28, 1919.

No. 454. December. Analyses of insecticides and fungicides. Pages 15. Distributed May 28, 1919.

No. 455. December. Feeding-stuffs. Pages 186. Distributed May 28, 1919.

No. 456. December. Control of a city milk supply. Robert S. Breed. Pages 10. Distributed May 28, 1919.

No. 457. December. Director's report for 1918. W. H. Jordan. Pages 25. Distributed June 12, 1919.

TECHNICAL BULLETINS.

No. 64. January. Microscopic study of bacteria and fungi in soil. H. J. Conn. Pages 20. Distributed June 1, 1919.

No. 65. December. Studies relating to milk. Pages 54. Distributed May 28, 1919.

CIRCULARS.

No. 54. Revised. Milking machines. Pages 5.

No. 56. January 1. Standardization of market milk. Dr. L. L. Van Slyke. Pages 9.

No. 57. February 20. Insect injuries of apple fruit. Bentley B. Fulton. Pages 15.

No. 58. (Condensed and reprinted from Technical Bulletin No. 49, pp. 31, 1916). Counting bacteria by means of the microscope. Robert S. Breed and James D. Brew. Pages 12.

PUBLICATIONS ISSUED DURING 1919.

BULLETINS.

No. 458. January. A test of commercial fertilizers for grapes. Fred E. Gladwin. Pages 24. Distributed June 12, 1919.

No. 459. March. Missing hills in potato fields: their effect upon the yield. F. C. Stewart. Pages 69. Figs. 3. Distributed June 12, 1919.
Popular edition, pages 5. Distributed December 1, 1919.

No. 460. May. Twenty years of fertilizers in an apple orchard. U. P. Hedrick and R. D. Anthony. Pages 95. Distributed December 1, 1919.

No. 461. June. Control of green apple aphis in bearing orchards. H. E. Hodgkiss. Pages 134. Plates 10. Distributed December 1, 1919.

No. 462. June. Seed tests made at the Station during 1918. I. Inspection of Agricultural Seeds. II. Voluntary examinations for correspondents. M. T. Munn. Pages 156. Distributed December 1, .1919.

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No. 464. July. A test of methods in pruning the Concord grapes in the Chautauqua grape belt. F. E. Gladwin. Pages 213. Plates 10. Distributed April 15, 1920.

No. 465. June. An experience in crop production. W. H. Jordan and G. W. Churchill. Pages 20. Distributed December 1, 1919.

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No. 74. December. Relation between lactic acid production and bacterial growth in the souring of milk. J. C. Baker, J. D. Brew, and H. J. Conn. Pages 24. Distributed June 15, 1920.

W. H. JORDAN.

NEW YORK AGRICULTURAL EXPERIMENT STATION,
GENEVA, N. Y., January 15, 1920.

REPORT

OF THE

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I. An experience in crop production.

REPORT OF THE DEPARTMENT OF AGRONOMY.

AN EXPERIENCE IN CROP PRODUCTION.*

W. H. JORDAN AND G. W. CHURCHILL.

SUMMARY.

1. The experience with crop production herewith recorded covers seventeen years of observation, and the data herein given may be regarded as a report of progress.

2. The experiment was extended thru four years of rotations of four years each, the crops being corn, oats, wheat and grass, in the order named. On four of the plats, covering the four methods of treatment, clover was included in the rotation, and on the other four plats, receiving entirely similar treatments, timothy was included in the rotation.

3. The points concerning which conclusions more or less definite may be drawn are the following:

The relative production of crops with farm manure, complete commercial fertilizer and acid phosphate which was supplemented by a small application of sodium nitrate.

A comparison of farm manure and complete commercial fertilizer.

The influence of clover as a factor in fertility.

Soil analysis as a means of measuring fertility.

The relative profitableness of the several methods of treatment.

4. The largest yield of crops, measured in terms of dry matter, was with farm manure altho this did not greatly exceed the production with complete commercial fertilizer. Both the farm manure and the complete commercial fertilizer produced approximately 56 per ct. more dry matter than the plats receiving no fertilizer. The plats receiving phosphoric acid with partial nitrogen and no potash produced about 33 per ct. more than the untreated plats.

5. The production of dry matter with the farm manure and complete commercial fertilizer was in the proportion of 118.3 for the former to 113.9 for the latter. If, however, allowance is made for

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the difference in yield of hay due to the fact that timothy sod was maintained for only one year, and comparison is made of the cereal crops produced, the relation of farm manure to commercial fertilizer is as 91 to 90 with clover in the rotation, and with timothy in the rotation as 84 to 89.

6. A comparison of the clover plats with the timothy plats shows that in the seventeen years there were produced of all crops 29,800 lbs. more of dry matter on the clover plats than on the timothy plats. If, however, we make the comparison on the basis of the cereal crops, the difference in favor of clover is 13,500 lbs. of dry matter. These results indicate considerable advantage from the use of clover.

7. A study of the yield of dry matter on the plats receiving no fertilizer, one with clover in the rotation and the other with timothy, shows that production was maintained on these plats as effectively with timothy as with clover in the rotation. The yields following the first rotation were maintained without any essential drop.

8. A comparison of the analysis of the soils on the several plats before and after seventeen years of cropping showed no differences or changes which gave either any indication of the effect of the unlike systems of treatment or of the unlike productivity of the plats at the end of the seventeen years.

9. A comparison of the results of the experiment on the basis of the cost of the fertilizers and the value of the crops shows that the cheapest increase of production was with acid phosphate combined with a minimum amount of nitrate of soda.

INTRODUCTION.

The general problem of crop production is exceedingly complex. Many factors, physical, chemical and biological in their nature, are immediately related to fertility and the aggregate influence of these factors determines whether any particular soil is fertile. Because of the complicated activities and influences originating in the soil, progress in the solution of soil problems has been very slow and in no phase of agricultural science has the development of well established knowledge been so unsatisfactory.

The study of soil problems has been prosecuted along two general lines, laboratory investigations and experience in the growth of

plants, either in forcing houses or in the fields. Laboratory research has been essential and useful in establishing certain fundamental facts, but has been inadequate for the determination of what would occur under field conditions. Forcing-house experiments have been enlightening in certain directions, but it is a matter of common observation that results in growing plants under glass have not been entirely comparable with those secured in field culture. In field experiments it has not been possible in most instances to determine either the absolute or relative influence of the various individual factors which influence plant growth. Moreover, the results of such experiments are determined by local conditions which may or may not be similar to the conditions of any other farm or region. The records reveal a great activity during many years in carrying on field experiments with fertilizers, of which there has been a very large number, but it is safe to assert that these experiments have established few principles or facts of general application, which would serve as a safe guide to an individual farmer in regulating his practice. It would appear that the maintenance of fertility is a local problem.

GENERAL CONDITIONS INVOLVED IN THE EXPERIMENTS.

The field experiment, the results of which are herewith discussed, was begun in the year 1896 and the data now presented cover the years from this beginning until and including 1913. The season of 1896 was devoted to determining the relative productiveness of each of the two halves of the field utilized. Beginning with 1897 a rotation of crops was followed: corn, oats, wheat and grass, with the exception that two crops of corn were grown in succession in 1897 and 1898. This means that the period thru which the experiment was conducted included four rotations covering 17 years of time. The area of the field selected for this work was twelve acres. This was divided into eight plats with a space of four feet dividing the plats, thus allowing approximately one acre and a half to each plat. The actual dimensions of the plats were 4 rods x 60 rods.

NOTE.—The chemical analyses involved in the data herewith reported were performed by E. B. Hart, E. L. Baker, M. P. Sweeney, and R. F. Keeler.

The following diagram shows the arrangement of the plats and the treatment which each plat received:

No. of plat.	ARRANGEMENT AND GENERAL TREATMENT OF THE PLATS.	
1.	Farm manure,	Clover in rotation.
2.	No fertilizer,	Clover in rotation.
3.	Partial fertilizer, P_2O_5 and minimum N.	Clover in rotation.
4.	Farm manure,	Timothy in rotation.
5.	Complete fertilizer, P_2O_5, K_2O and maximum N.	Clover in rotation.
6.	No fertilizer,	Timothy in rotation.
7.	Partial fertilizer, P_2O_5 and minimum N.	Timothy in rotation.
8.	Complete fertilizer, P_2O_5, K_2O and maximum N.	Timothy in rotation.

The following points should be noted:

1. With four of the plats clover was used in the rotation and on the other four plats, timothy.
2. Two plats received no fertilizer whatever during the seventeen years.
3. Two plats received phosphoric acid with a minimum amount of nitrogen.
4. Two plats received what is known as a complete fertilizer, namely, nitrogen, phosphoric acid and potash.
5. Two plats received farm manure.

In 1897 winter vetch was sown in the corn on Plats 1, 2, 3 and 5 at the last cultivation. The seed catch was good, the plants did not winter kill and much growth was made in the spring before the second crop of corn.

In 1898 crimson clover was sown on Plats 1, 2, 3 and 5 at time of last cultivation. The seed catch was good, the autumn growth was satisfactory, but the plants winter-killed largely.

The fertilizers of all kinds were applied to the corn and the wheat, no application being made to the oats and grass.

The yearly and total quantities of fertilizers applied to the several plats during the seventeen years are given for each plat in Table I.

TABLE I.— QUANTITIES OF FERTILIZERS APPLIED EACH YEAR DURING THE EXPERIMENTS.

YEAR.	PLATS 1 and 4.	PLATS 3 AND 7.				PLATS 5 AND 8.			
	Farm manure.	Acid Phosphate.	Dried blood.	Nitrate soda.	Muriate potash.	Acid Phosphate.	Dried blood.	Nitrate soda.	Muriate potash.
	Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
1897.....	33,325	450	450	75	75	450	525	225	300
1898.....	30,000	600	......	150	......	600	525	225	300
1899.....	30.000	300	150	50	......	300	750	225	90
1900.....	......	......	......	......	......	......	......	......	....
1901.....	......	......	......	......	......	......	......	......	....
1902.....	30,000	300	......	150	......	300	360	300	150
1903.....	30,000	200	100	25	......	200	500	100	70
1904.....	......	......	......	......	......	......	......	......	....
1905.....	......	......	......	......	......	......	......	......	....
1906.....	30,000	300	......	200	......	300	700	200	225
1907.....	30,000	300	......	300	......	300	500	300	150
1908.....	......	......	......	......	......	......	......	......	....
1909.....	......	......	......	......	......	......	......	......	....
1910.....	30,000	300	250	50	......	300	700	200	225
1911.....	30,000	300	250	50	......	300	700	200	225
1912.....	......	......	......	......	......	......	......	......	....
1913.....	......	......	......	......	......	......	......	......	....
Totals....	273,325	3,050	1,200	1,050	75	3,050	5,260	1,975	1,735

RELATIVE YIELD OF THE SEVERAL PLATS UNDER DIFFERENT METHODS OF TREATMENT DURING THE ENTIRE PERIOD OF THE EXPERIMENT, INVOLVING FOUR ROTATIONS.

PRODUCTION OF CROPS AS HARVESTED.

The yield of the several plats involved in this experiment is measured in two ways: first, by the weights of the crops as harvested and, second, by the amount of dry matter contained in the crops. The latter measurement is the one which should be chiefly considered because the dry matter is a fundamental measurement of the production of crops either for human food or for food for animals. Table II gives the weights of the crops of each kind for each year as well as the totals for the four rotations. It should be explained that in 1897, 1898 and 1906 two rows out of the twenty-two rows in each plat were allowed to stand and ripen for husking while the other twenty rows were cut before complete ripeness and stored in a silo. In order to compute the total yield of fresh corn for silage in those years, the weights of corn in the silage condition were increased for each plat by one-tenth. In 1902, all the corn was cut and stacked in the field. The probable weight in the fresh

TABLE II.— CROPS AS HARVESTED.

CORN CUT FOR SILAGE.

YEAR.	PLATS. 1.	2.	3.	4.	5.	6.	7.	8.
	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.	Lbs.
1897[1]	35,120	28,793	33,368	34,497	38,612	31,970	33,970	37,760
1898[1]	37,638	26,469	31,126	32,486	34,624	25,421	29,260	32,644
1902[2]	17,620	12,528	14,525	14,466	16,504	11,273	13,283	15,634
1906[1]	25,355	19,700	21,106	23,081	25,219	17,464	20,978	25,725
1910	45,416	27,574	38,059	42,286	38,702	29,790	33,916	45,697
Total	161,149	115,064	138,184	146,816	153,661	115,918	131,407	157,460
Corrected figures[3]	187,607	130,335	158,718	166,481	176,325	131,820	149,216	183,594
Increase over check	57,272	....	28,383	34,661	45,989	....	17,395	51,774

OAT STRAW.

YEAR.	1.	2.	3.	4.	5.	6.	7.	8.
1899	3,337	2,742	3,735	3,549	3,966	2,906	3,224	3,463
1903	1,751	1,790	1,969	2,417	2,006	1,724	2,073	2,506
1907	3,216	2,192	3,086	3,197	3,082	2,225	2,510	2,851
1911	2,445	1,120	2,014	2,518	2,138	1,342	1,768	2,470
Total	10,749	7,844	10,804	11,681	11,192	8,197	9,575	11,290
Increase over check	2,905	.....	2,960	3,484	3,348	.....	1,378	3,093

OATS — GRAIN.

YEAR.	1.	2.	3.	4.	5.	6.	7.	8.
1899	3,195	2,386	3,301	2,959	3,148	2,548	2,612	3,053
1903	814	749	780	728	778	626	751	924
1907	2,416	1,568	2,224	2,233	2,342	1,535	1,850	1,900
1911	1,940	1,262	1,942	2,216	2,080	1,450	1,832	2,160
Total	8,365	5,965	8,247	8,136	8,348	6,159	7,045	8,037
Increase over check	2,400	....	2,282	1,977	2,383	....	886	1,878

WHEAT — STRAW

YEAR.	1.	2.	3.	4.	5.	6.	7.	8.
1899	3,395	1,258	2,340	3,426	3,506	1,590	2,020	3,185
1908	4,797	2,346	3,964	3,954	4,563	2,302	3,665	3,962
1912	5,127	2,297	4,150	5,367	5,084	2,032	3,976	3,225
Total	13,319	5,901	10,454	12,747	13,153	5,924	9,661	10,372
Increase over check	7,418	....	4,553	6,823	7,252	....	3,737	4,448

WHEAT — GRAIN

YEAR.	1.	2.	3.	4.	5.	6.	7.	8.
1899	2,670	1,022	1,890	2,950	2,940	1,380	1,780	2,470
1908	3,200	1,788	3,120	2,838	3,540	1,840	2,750	2,958
1912	3,174	1,246	2,211	3,030	3,155	1,086	2,202	3,270
Total	9,044	4,056	7,221	8,818	9,635	4,306	6,732	8,698
Increase over check	4,988	....	3,165	4,512	5,519	....	2,426	4,392

[1] Ten-elevenths of the crop.
[2] Weight of air dry crop.
[3] Figures for 1897, 1898 and 1906 increased one-tenth. Fresh crop for 1902 estimated by multiplying the dry matter by four.

TABLE II — (*Continued*).

HAY

YEAR.	PLATS.							
	1.	2.	3.	4.	5.	6.	7.	8.
	Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
1901	6,281	2,917	4,828	7,388	5,028	3,406	4,586	5,502
1905	12,955	8,364	10,823	5,841	11,250	3,104	3,163	2,796
1909	8,005	2,685	4,756	8,132	6,006	2,633	4,004	4,357
1913	7,609	4,132	6,001	9,397	7,220	4,204	6,581	6,520
Total	34,850	18,098	26,408	30,758	29,504	13,347	18,334	19,175
Increase over check	16,752	....	8,310	17,411	11,406	....	4,987	5,828

BARLEY — STRAW

1904	3,533	2,650	3,387	2,998	3,524	2,416	2,551	3,579
Increase over check	883	....	737	582	874	....	135	1,163

BARLEY — GRAIN

1904	1,902	1,242	1,484	1,615	1,867	1,209	1,413	1,939
Increase over check	660	....	242	406	625	....	204	730

condition is computed by multiplying the pounds of dry matter by four, as 25 per ct. was the general average for dry matter in corn harvested for silage. The detailed figures in Table II are followed by a summary (Table III) showing the total yields of

TABLE III.— SUMMARY OF YIELDS OF CROP AS HARVESTED DURING FOUR ROTATIONS COVERING A PERIOD OF 17 YEARS.

KIND OF CROP.	Plat 1.	Plat 2.	Plat 3.	Plat 4.	Plat 5.	Plat 6.	Plat 7.	Plat 8.
Corn for silage	187,607	130,335	158,718	166,481	176,325	131,820	149,216	183,599
Oat, straw	10,749	7,844	10,804	11,681	11,192	8,197	9,575	11,290
Oat, grain	8,365	5,965	8,247	8,136	8,348	6,159	7,045	8,037
Wheat, straw	13,319	5,901	10,454	12,747	13,153	5,924	9,661	10,372
Wheat, grain	9,044	4,056	7,221	8,818	9,635	4,306	6,732	8,698
Barley, straw	3,533	2,650	3,387	2,998	3,524	2,416	2,551	3,579
Barley, grain	1,902	1,242	1,484	1,615	1,867	1,209	1,413	1,939
Hay	34,850	18,098	26,408	30,758	29,504	13,347	18,334	19,175

each crop for the entire period and also (Table IV) giving the increase in yield over the check plats for the entire period.

As before stated the latter figures are the more significant, because the crops as harvested carry unknown quantities of water.

TABLE IV.— SUMMARY OF INCREASES OF YIELDS OVER THE CHECK PLATS FOR THE FOUR ROTATIONS COVERING A PERIOD OF 17 YEARS.

KIND OF CROP.	Plat 1.	Plat 2.	Plat 3.	Plat 4.	Plat 5.	Plat 6.	Plat 7.	Plat 8.
Corn, silage	57,272	....	28,383	34,661	45,698	....	17,395	51,774
Oat, straw	2,905	....	2,960	3,484	3,348	....	1,378	3,093
Oats, grain	2,400	....	2,282	1,977	2,383	....	886	1,878
Wheat, straw	7,418	....	4,553	6,823	7,252	....	3,737	4,448
Wheat, grain	4,988	....	3,165	4,512	5,519	....	2,426	4,392
Barley, straw	883	....	737	582	874	....	135	1,163
Barley, grain	660	....	242	406	625	....	204	730
Hay	16,752	....	8,310	17,411	11,406	....	4,987	5,828

PRODUCTION OF DRY SUBSTANCE.

In Table V are given the weights of dry substance produced on each plat during the entire period of four rotations. In order to determine the yield of dry substance, very large samples were taken from each plat at each harvest, either by cutting many hills of corn distributed over the entire area of each plat or by taking samples of straw and grain at the time the grain was threshed or by selecting samples of hay at the time of storage in the barn. These samples were re-sampled for preparation for laboratory uses, the large samples being again weighed at the time of the selection of the smaller samples. The difficulty of absolute accuracy in such sampling is well recognized, but it is believed that the methods employed precluded serious errors.

A study of the following table reveals certain facts worthy of some attention.

TABLE V.— YIELD OF DRY SUBSTANCE UNDER THE SEVERAL METHODS OF TREATMENT.

Number of plat and treatment.	Corn.	Oats.	Wheat.	Barley.	Hay.	Total.
	Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
1. Farm manure	48,659.9	17,036.3	20,636.5	4,973.3	27,061.8	118,367.8
2. No fertilizer	32,998.1	12,422.4	9,059.8	3,555.1	14,818.4	72,853.8
3. Partial fertilizer	41,273.0	17,184.7	15,563.5	4,381.1	21,689.6	100,091.9
4. Farm manure	42,932.0	17,898.2	19,311.8	4,206.1	25,211.4	109,559.8
5. Complete fertilizer	45,442.5	17,451.0	20,418.9	4,882.0	23,722.8	111,917.2
6. No fertilizer	34,615.2	12,802.8	9,198.9	3,343.4	10,649.6	70,619.9
7. Partial fertilizer	40,731.6	14,941.2	14,727.4	3,670.7	14,921.5	88,932.4
8. Complete fertilizer	50,489.0	17,191.3	17,251.5	5,013.0	16,353.1	106,297.9
Total	337,141.6	128,937.9	126,168.3	33,964.7	154,428.2	....

Plats 1, 2, 3 and 5 with clover in the rotation.
Plats 4, 6, 7 and 8 with timothy in the rotation.

The most fundamental consideration is the great variation in production, due to the different methods of treatment. The plats receiving farm manure and complete commercial fertilizer produced approximately 56 per ct. more dry matter than the plats receiving no fertilizer. The plats receiving phosphoric acid with partial nitrogen and no potash produced about 33 per ct. more than the untreated plats. In considering these figures, it should be kept in mind that the crops were produced under a system of rapid rotation and, in case of four of the plats fine crops of clover were grown in each rotation so that there was turned under on these plats an excellent clover sod together with more or less second growth in certain years. Moreover, in the year 1910 all the plats received one ton of burned lime per acre, previously slaked. If any evidence is necessary, such figures should completely dispose of the doctrine somewhat prevalent at one time that the chief function of commercial fertilizers was palliative in character and that with proper rotation and soil treatment desirable production could be maintained. Another interesting and important fact displayed is the relative productivity of the several crops entering into the experiment. The figures show that the growth of dry substance in corn is over twice that in either oats or wheat (counting the yield of barley as part of the wheat), or even of hay — notwithstanding the fact that the crops of the cereal grains and hay were considered very satisfactory. This is a fact to which the dairy farmer should give careful consideration in planning to secure the largest amount possible of available food for milk production.

THE RELATIVE EFFICIENCY OF FARM YARD MANURE AND CHEMICAL FERTILIZERS.

A question much discussed, especially thruout the Eastern States, is the maintenance of fertility by the use of commercial fertilizers. The figures obtained in this experiment should serve as a partial answer to this question. A comparison of the results of these two methods is found in Table VI.

If we accept the figures on their face value, the superiority of farm manure must be conceded, altho comparison does not show a large difference in results from the two methods of treatment. In considering these figures, it should be borne in mind that altho

the amount of farm manure applied did not exceed what is regarded as a liberal quantity, doubtless the quantities of the valuable plant food elements present in the farm manure were much above those applied in the complete commercial fertilizer.

TABLE VI.— COMPARATIVE YIELD OF DRY MATTER, ALL CROPS, WITH FARM MANURE AND "COMPLETE" COMMERCIAL FERTILIZER.

	Total yield dry substance with clover in rotation	Total yield dry substance with timothy in rotation
	Lbs.	*Lbs.*
Farm manure	118,367.8	109,559.8
"Complete" fertilizer	113,917.2	106,297.9
	4,450.6	3,262.9

In Table I it is seen that on Plats 1 and 4 thirty thousand pounds of farm manure were applied to each plat twice in each rotation.

Analyses were made of this manure for five consecutive years, with the following results:

TABLE VII.— COMPOSITION OF FARM MANURE USED IN EXPERIMENT.

	Water.	Nitrogen.	Phosphoric acid P_2O_5.	Potash K_2O.	Lime Ca.O.
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
	75.9	.386	.357	.339	.441
	77.8	.340	.222	.564	.317
	76.3	.486	.531	.784	.964
	76.7	.584	.500	.917	....
	71.4	.390	.398	.744	.744
Average	....	.437	.401	.673	.611

It appears from these figures that on the average the following quantities of nitrogen, phosphoric acid and potash were applied every second year to Plats 1 and 4.

Nitrogen.	Phosphoric acid.	Potash.	Lime.
131 lbs.	120 lbs.	202 lbs.	183 lbs.

These are quantities much larger than were supplied in the most liberal application of commercial fertilizer.

Another factor to be considered is that the plats were allowed in grass but one year, so that the timothy plats were placed at a great disadvantage because of the well known fact that a timothy sod should be maintained more than one year in order to get maximum results. If, therefore, we subtract from the total quantities of dry matter produced under the two systems the dry matter contained in clover hay and timothy hay we are then able to compare the relative influence of farm manure and a complete commercial fertilizer in the production of the cereal grains. The figures are somewhat discordant. The farm manure appears to have produced somewhat better results on the clover plats but induced a yield on the timothy plats inferior to that of the commercial fertilizers. This is shown in Table VIII.

TABLE VIII.— FARM MANURE VS. CHEMICAL FERTILIZER FOR GRAIN CROPS.

	Total yield dry substance from grain crops with clover in the rotation.	Total yield dry substance from grain crops with timothy in the rotation.
	Lbs.	*Lbs.*
Farm manure	91,306.0	84,348.4
"Complete" fertilizer	90,194.4	89,944.8
	+1,111.6	—5,596.4

As this experiment so far conducted covers a period of seventeen years, these results have some weight in considering the efficiency of the two methods of maintaining fertility.

CLOVER AS A FACTOR IN FERTILITY.

Scientific investigation has shown conclusively that under certain conditions the leguminous plants are able to acquire atmospheric nitrogen. Basing arguments upon this fact, agricultural teachers have advised the farmers that clover is a very important factor in maintaining the fertility of the soil. Conclusive measurements of this value might not easily be discovered in the records of investigation. The claims for clover rest upon its acquisition of nitrogen, its extensive root development thereby enlarging the area from which plant food may be derived and also furnishing a relatively

large amount of organic matter to the soil when a clover sod is turned under. One of the objects of this experiment now under discussion was to make observation on the influence of clover on fertility. It is to be noted that during a period of seventeen years four of the plats in question have been seeded to clover and four plats to timothy. Fairness requires the statement that the timothy plats have not been wholly free from leguminous plants of one kind or another — red clover to a slight extent and one or more species of Trifolium in addition. Moreover, as only one year of cutting of timothy was permitted, the cereal crops have had a certain advantage because the crops from the timothy plats were not nearly as large as the crops from the clover plats. Nevertheless, there has been turned under a fine clover sod on four of the plats during each of the rotations. It was expected that the advantage of the clover would be seen particularly on the check plats and on the plats receiving a very limited amount of nitrogen in the commercial fertilizer. Table IX summarizes the relative yields on

TABLE IX.— EFFECT OF CLOVER ON CROPS IN ROTATION.

	Yield dry substance from the various methods of treatment with clover in the rotation.	Yield dry substance from the various methods of treatment with timothy in the rotation.	Differences: excess with clover. All crops.	Differences: excess with clover. Cereal crops.
	Lbs.	*Lbs.*	*Lbs.*	*Lbs.*
Farm manure	118,367.8	109,559.8	8,808.0	6,957.6
Complete fertilizer	111,917.2	106,297.9	5,619.3	451.5
Partial fertilizer	100,091.9	88,932.4	11,159.5	4,391.4
No fertilizer	72,853.8	70,619.9	2,233.9	—1,934.9
Total difference	....	....	27,820.7	9,865.6

the plats with clover in the rotation and on the plats with timothy in the rotation.

If we were to accept these figures as they stand for all the crops, the claims for the value of clover in a rotation would be justified. In view of the fact that the yield of hay on the timothy plots was not as large as would have been true had the rotation continued for five years, giving a second year's growth of the timothy, it is probably fairer to judge the influence of the clover by its effect upon the yield of the cereal grains. If, therefore, we subtract from the total figures the yields of hay, the excess of the yield of the

clover plats (see last column of Table IX) is considerably reduced in all cases and with the check plats the larger yield is seen to be on the timothy plats. Perhaps a severer test of the value of the clover is to be seen in its influence upon the maintenance of crop production on the check plats. These plats produced crops during four rotations, covering seventeen years, and the figures given in Table X show the total value during these periods.

TABLE X.— RATE OF YIELD OF DRY SUBSTANCE IN A SERIES OF YEARS WITH NO FERTILIZER.

PLAT 2 — CLOVER IN ROTATION.

Rotations 4 years each.	Corn.	Oats.	Wheat.	Hay.	Total.
	Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
First	7,887.7	4,436.7	2,060.6	2,562.0	16,947.0
Second	5,075.8	2,213.1	3,555.1	7,029.9	17,837.9
Third	6,024.4	3,553.5	3,046.0	1,834.7	14,458.6
Fourth	6,135.2	2,219.1	3,194.6	3,211.8	14,760.7

PLAT 6 — TIMOTHY IN ROTATION.

Rotations 4 years each.	Corn.	Oats.	Wheat.	Hay.	Total.
	Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
First	7,677.0	4,690.6	2,578.4	2,757.8	17,703.8
Second	4,922.3	2,084.9	3,343.3	2,314.8	12,665.3
Third	5,660.4	3,415.9	3,793.1	2,088.5	14,957.9
Fourth	7,215.1	2,561.4	2,827.4	3,488.4	16,092.3

The figures in Table X are somewhat surprising. It appears that after the first rotation the yield of dry matter was practically maintained on a level with the exception of a crop in the timothy plats in the second rotation. The yield in the fourth rotation is found to have been practically as large as in any previous period excepting the first. It is well known that the soil on the Experiment Station farm has large potential fertility, but it is rather surprising that without the use of any fertilizer no decrease in productivity was observed during the last twelve years of cropping. The outstanding fact in this connection is that on the timothy plats the crop production was maintained as efficiently as on the clover plats.

SOIL ANALYSIS AS A MEANS OF MEASURING FERTILITY.

This long continued experiment in cropping land under different treatments has given an opportunity to make observations on the value of soil analysis as a means of measuring soil fertility. When the experiment was begun, in 1897, samples of soil were taken from the several plats, one set of samples representing the first six inches of soil and another set the second six inches. The samples were at that time analyzed, the total nitrogen and the percentage of other ingredients soluble in 1.2 sp. gr. strength hydrochloric acid being determined. At the end of the four rotations, covering seventeen years' cropping, samples were again taken from the several plats, one set representing the first seven inches in depth of soil and another set the second seven inches. In the latter case the plats were divided into two halves and separate samples were taken from the east and west halves. These latter samples were analyzed by methods which determined the total percentages present of the various ingredients and the first set of samples taken in 1897 were re-analyzed by a similar method. Table XI, XII and XIII give the results of these analyses.

TABLE XI.—SOIL NITROGEN IN SOIL OF 12 ACRE FIELD.

Old sample, first six inches; new sample, first seven inches.

No. plat.	Total nitrogen.						Treatment.
	Old sample.		New samples, after 17 years' cropping.			Variation.	
	1st analysis.	2nd analysis.*	East.	West.	Av. both halves.		
	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	
1.	.191	.169	.190	.167	.178	.009	Clover, farm manure.
2.	.158	.158	.162	.121	.142	—.016	Clover, check.
3.	.197	.203	.243	.127	.185	—.018	Clover, P and min. N.
4.	.195	.197	.209	.146	.178	—.019	Timothy, farm manure.
5.	.194	.196	.175	.138	.157	—.039	Clover, P, K and max. N.
6.	.224	.217	.195	.122	.159	—.058	Timothy, check.
7.	.201	.208	.212	.142	.177	—.031	Timothy, P and min. N.
8.	.257	.257	.237	.156	.197	—.060	Timothy, P, K and max. N.

* After 16 years' storage.

Old sample, 7 to 12 inches; new sample, 8 to 14 inches.

1.	.106	.108	.109	.092	.100	—.008	Clover, farm manure.
2.	.089	.092	.094	.073	.084	—.008	Clover, check.
3.	.111	.132	.177	.107	.142	.010	Clover, P and min. N.
4.	.135	.142	.163	.088	.126	—.016	Timothy, farm manure.
5.	.159	.160	.113	.083	.098	—.062	Clover, P, K and max. N.
6.	.150	.145	.141	.092	.127	—.018	Timothy, check.
7.	.103	.104	.149	.081	.115	.009	Timothy, P and min. N.
8.	.164	.151	.176	.096	.136	—.015	Timothy, P, K and max. N.

TABLE XII.— PHOSPHORUS IN SOIL OF 12 ACRE FIELD.
Old sample, first six inches; new sample, first seven inches.

No. plat.	P_2O_5					
	Old sample.	New samples, after 17 years' cropping.			Variation.	Treatment.
		East half.	West half.	Av. both halves.		
	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	
1	.112	.159	.121	.140	.028	Clover, farm manure.
2	.113	.133	.126	.130	.017	Clover, check.
3	.146	.202	.123	.163	.017	Clover, P and min. N.
4	.135	.179	.131	.155	.020	Timothy, farm manure.
5	.132	.136	.128	.132	....	Clover, P, K and max. N.
6	.157	.171	.127	.149	—.008	Timothy, check.
7	.151	.177	.127	.152	.001	Timothy, P and min. N.
8	.158	.206	.124	.165	.007	Timothy, P, K and max. N.
	Old sample, 7 to 12 inches; new sample, 8 to 14 inches.					
1	.093	.136	.114	.125	.032	Clover, farm manure.
2	.104	.120	.131	.125	.021	Clover, check.
3	.129	.184	.124	.154	.025	Clover, P and min. N.
4	.112	.163	.127	.145	.033	Timothy, farm manure.
5	.132	.123	.129	.126	—.006	Clover, P, K and max. N.
6	.140	.166	.124	.145	.005	Timothy, check.
7	.127	.161	.124	.142	.015	Timothy, P and min. N.
8	.139	.177	.122	.150	.011	Timothy, P, K and max. N.

TABLE XIII.— CALCIUM OXIDE IN SOIL OF 12 ACRE FIELD.
Old sample, first six inches; new sample, first seven inches.

No. Plat.	CaO.				
	Old sample.	New samples, after 17 years' cropping.		Average both halves.	Variation.
		East half.	West half.		
	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.
1	1.16	.95	1.25	1.10	—.06
2	1.16	.98	1.43	1.20	.04
3	1.16	1.07	1.49	1.28	.12
4	1.05	1.02	1.43	1.22	.17
5	1.13	.96	1.28	1.12	—.01
6	1.26	1.07	1.43	1.25	—.01
7	1.15	1.08	.86	.97	—.18
8	1.37	1.17	.73	.95	—.42

The figures are worthy of careful consideration. Much difference of opinion has existed as to the value of soil analysis in a study of the fertility of any given area of soil. It has been strongly held,

probably by a majority of those who have carefully considered this question, that a soil analysis is not competent to measure available plant food or to differentiate between the fertility of two apparently unlike soils or soils having greatly unlike treatment. Others have held that soil analysis is important in indicating potential fertility. A close study of these figures which give the percentages of nitrogen, phosphorus and calcium oxides in the soil from these several plats reveals no relation of these percentages to the productivity of the plats at the end of the experiment; nor do the figures indicate appreciable changes in the composition of the soils after seventeen years of greatly unlike treatment in the application of fertilizing materials. It might be expected that plats 1, 4, 5 and 8 would show not only in increased percentages of nitrogen, or at least would not show so large a decrease in the percentages as plats 2, 3, 6 and 7. A close examination of the figures does not show that the composition of the several soils or the changes in composition have any relation to treatment or productivity. Those who have carefully analyzed the possibilities of soil analysis will not be surprised at this result. The weight of soil in an acre to the depth of a foot is from three to four million pounds, according to the character of the soil, and in order to affect the composition of this weight of material sufficiently to be revealed through present methods of analysis would require the removal of very large quantities of soil compounds. We have yet much to learn concerning the laboratory examination of soil as a means of measuring soil fertility.

THE BUSINESS SIDE OF THE EXPERIMENT.

It has been quite customary in discussing field experiments with fertilizers to measure the results of the different methods of treatment by the market value of the increase of crops which the fertilizers produce. Such experiments, if expressed only in terms of market values, are merely tests of business methods. The final figures are merely an expression of profit or loss, and with a given system of fertilization profit might be realized one year and loss the next, or profit might be secured in one locality and loss in another. The conclusions regarding an experiment formulated in this way do not express anything fundamental. It is possible, however, to study results as expressed in market values so as to get a comparison of

relative profits under a given set of circumstances, but the figures displayed may have a very limited application.

Since the experiment under consideration was begun, there has been a very great change in market prices both of fertilizers and crops. In Table XIV there is stated as nearly as can be estimated the prices of fertilizing materials and crops during the main portion

TABLE XIV.— RANGE OF PRICES DURING EXPERIMENT.

FERTILIZER PRICES.	Ton prices during experiment.	Present prices per ton.
Acid phosphate	$14.00	$26.00
Dried blood, 10% N	40.00	80.00
Nitrate soda, 15% N	54.00	90.00
Muriate potash, 50%	45.00	225.00
Stable manure	2.00	4.00
PRICES OF PRODUCE.	**Prices during experiment per ton.**	**Present prices per ton.**
Silage	$3.00	$6.00
Hay	10.00	20.00
Oat straw	8.00	12.00
Oat grain	*.40	*.70
Wheat straw	6.00	12.00
Wheat grain	*1.00	*2.50
Barley straw	5.00	10.00
Barley grain	*1.00	*1.00

* Price per bushel.

of the time covered by the experiment and also prices at the present time. There is given in Tables XV to XVIII the increase in crops from various methods of treatment, both of the crop as harvested and of the dry substance, the cost of the fertilizers and the values of the crops. On the basis of these figures there is calculated the excess of crop values over fertilizer costs and the cost of one pound of increase of both total and dry substance on all of the plats.

TABLE XV.— INCREASES OF PRODUCTION DUE TO FERTILIZERS ON PLATS WITH CLOVER IN ROTATION.

	PLAT 1.		PLAT 3.		PLAT 5.	
	Crops as harvested.	Dry substance.	Crops as harvested.	Dry substance.	Crops as harvested.	Dry substance.
	Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
Corn silage	57,289	15,661	28,380	8,275	46,006	12,444
Oat straw	2,905	2,535	2,960	2,747	3,348	2,900
Oat grain	2,400	2,079	2,282	2,015	2,383	2,128
Wheat straw	7,418	7,125	4,533	3,629	7,252	6,455
Wheat grain	4,988	4,451	3,165	2,875	5,579	4,904
Barley straw	883	821	737	605	874	767
Barley grain	660	597	242	221	625	560
Hay	16,752	12,243	8,310	6,871	11,406	8,904
Totals	....	45,512	....	27,238	....	39,063

TABLE XVI.— COMPARISON OF FERTILIZER COSTS, CROP INCREASES AND VALUES ON PLATS WITH CLOVER IN ROTATION.

	PLAT 1.		PLAT 3.		PLAT 5.	
	Former value crops.	Present value crops.	Former value crops.	Present value crops.	Former value crops.	Present value crops.
Corn silage	$85.92	$171.87	$42.51	$85.14	$69.00	$178.00
Oat straw	11.62	17.43	11.85	17.76	13.39	20.08
Oat grain	30.20	52.56	28.52	49.97	29.80	52.19
Wheat straw	22.25	44.50	13.60	27.20	21.75	43.50
Wheat grain	83.13	207.82	52.75	131.87	93.00	242.50
Barley straw	2.20	4.40	1.84	3.68	2.18	4.36
Barley grain	9.44	9.44	3.52	3.52	8.96	8.96
Hay	83.76	167.52	41.15	82.50	57.03	114.06
Value crops	328.52	675.54	195.79	40.144	295.11	663.65
Fertilizer cost	267.32	544.00	70.29	145.73	220.69	618.26
Excess	61.20	131.54	125.50	255.71	74.42	45.39
Fertilizer cost of 1 lb. dry substance	.587¢	1.195¢	.258¢	.535¢	.565¢	1.583¢

TABLE XVII.— INCREASE IN PRODUCTION DUE TO FERTILIZERS ON PLATS WITH TIMOTHY IN ROTATION.

	PLAT 4.		PLAT 7.		PLAT 8.	
	Crops as harvested.	Dry substance.	Crops as harvested.	Dry substance.	Crops as harvested.	Dry substance.
	Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
Corn	34,661	8,317.1	17,395	6,116.4	51,774	15,513.0
Oat straw	3,484	3,343.4	1,378	1,302.4	3,092	2,732.7
Oat grain	1,977	1,751.9	886	835.9	1,878	1,655.9
Wheat straw	6,823	6,163.9	3,737	3,354.2	4,448	4,125.5
Wheat grain	4,512	3,949.5	2,426	2,074.6	4,392	3,927.6
Barley straw	348	496.	204	187.5	1,163	1,014.7
Barley grain	406	365.8	135	79.8	730	654.9
Hay	17,411	14,561.8	4,987	4,271.8	5,829	5,703.5
Totals	......	38,950.1	......	18,322.6	......	35,387.8

TABLE XVIII.— COMPARISON OF FERTILIZER COSTS AND CROP VALUES ON PLATS WITH TIMOTHY IN ROTATION.

	PLAT 4.		PLAT 7.		PLAT 8.	
	Former value crops.	Present value crops.	Former value crops.	Present value crops.	Former value crops.	Present value crops.
Corn	$51.99	$103.98	$26.09	$52.18	$77.67	$155.34
Oat straw	13.92	20.88	5.51	8.35	12.37	18.55
Oat grain	24.72	43.29	11.04	19.32	23.48	41.09
Wheat straw	20.46	40.92	11.21	22.42	13.34	26.68
Wheat grain	75.00	187.50	40.40	101.00	73.20	183.00
Barley straw	.87	1.74	.34	.68	2.90	5.80
Barley grain	5.60	5.60	2.91	2.91	10.40	10.40
Hay	87.00	174.00	24.93	49.86	29.15	58.30
Value crops	279.56	577.91	142.53	256.72	242.51	599.16
Fertilizer cost	267.32	534.	70.39	145.73	220.69	618.26
Excess value crops	12.24	43.91	72.14	80.99	21.82	—19.10
Fertilizer cost, 1 lb. dry substance	.686¢	1.372¢	.384¢	.795¢	.623¢	1.747¢

It is entirely clear from the foregoing figures that the cheapest increase of production was secured by the use of acid phosphate, supplemented by a small proportion of sodium nitrate. Unquestionably, with this method of treatment the additional

yield of crops cost much less than the sum for which they could have been purchased either at former prices or at the prices now prevailing. Whether or not the increased yield from the use of the complete fertilizer with a maximum amount of nitrogen would be profitable would depend upon circumstances. It is very difficult to draw conclusions as to business results which would be generally applicable. It is certain that the returns from the use of farm manures indicate that this fertilizer was worth approximately the prices named.

CHANGE IN THE EXPERIMENTAL PLAN.

Beginning with the year 1914, each of the eight plats involved in the first seventeen years of the experiment was divided into two, making sixteen plats in all. This was done in order to study the results from the use of raw ground phosphate and to get additional information concerning the use of nitrogenous fertilizers. There follows the general scheme under which the experiment is now being conducted.

PLAN OF FERTILIZATION FOLLOWING THE YEAR 1913

12 ACRE FIELD

Plat	Fertilizer	Crops
1 a.	15,000 pounds manure *twice* in rotation — corn, wheat.	
1 b.	15,000 pounds manure once in rotation — wheat.	
2 a.	Raw ground phosphate, 300 pounds — corn, wheat.	
2 b.	Nothing.	
3 a.	Acid phosphate, 300 pounds. Dried blood, 175 pounds, nitrate soda, 50 pounds	corn, wheat.
3 b.	Acid phosphate, 300 pounds. Dried blood, 350 pounds, nitrate soda, 100 pounds	corn, wheat.
4 a.	15,000 pounds manure, *twice* in rotation — corn, wheat.	
4 b.	15,000 pounds manure, *once* in rotation — wheat.	
5 a.	Raw ground phosphate, 300 pounds. Dried blood, 350 pounds, nitrate soda 100 pounds, muriate potash, 100 pounds.	corn, wheat.
5 b.	Acid phosphate, 300 pounds. Dried blood, 350 pounds, nitrate soda, 100 pounds, muriate potash, 100 pounds.	corn, wheat
6 a.	Raw ground phosphate, 300 pounds.	
6 b.	Nothing.	
7 a.	Acid phosphate, 300 pounds. Dried blood, 175 pounds, nitrate soda, 50 pounds	corn, wheat.
7 b.	Acid phosphate, 300 pounds. Dried blood, 350 pounds, nitrate soda, 100 pounds	corn, wheat.
8 a.	Raw ground phosphate, 300 pounds. Dried blood, 350 pounds, nitrate soda, 100 pounds, muriate potash, 100 pounds.	corn, wheat.
8 b.	Acid phosphate, 300 pounds. Dried blood, 350 pounds, nitrate soda, 100 pounds, muriate potash, 100 pounds.	corn, wheat

REPORT

OF THE

Department of Animal Industry.

W. P. WHEELER, *First Assistant (Animal Industry).*

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I. Studies relating to calcium metabolism.

REPORT OF THE DEPARTMENT OF ANIMAL INDUSTRY.

STUDIES RELATING TO CALCIUM METABOLISM.*

W. P. WHEELER.

PREFACE.

The experiments which supplied the data drawn upon for this bulletin were made mostly a number of years ago.

Without the careful and dependable help of Mr. P. F. O'Neill, and later of Mr. John Conolly, the larger part of the work could not have been done. For some of the earlier feeding trials the intended chemical work was completed in large part along with the other work, but usually only enough could be done to control the feeding and find the general trend of results. For the larger number of feeding trials, analyses of ash, shells and a few other samples were made by Mr. O. B. Winter who did much work in the matter of testing out methods of analysis, particularly those for strontium estimation, and in adapting them to these particular samples. On samples from some of the later feeding, considerable analytical work, with variations of method, was done by Mr. R. F. Keeler. Analyses were also made earlier by Mr. A. W. Clark and by Mr. A. K. Burke.

Whatever value the results reported may have is due very largely to the careful help of these men, both with original analyses and in verifying work previously done. It is a matter of much satisfaction that separate determinations, in certain identical samples of significance, made after intervals of several years by different analysts, using somewhat different methods, gave consistent results in exceptionally close conformity.

SUMMARY.

With all farm animals (as well as man) calcium is found in the body in greater quantity than any other mineral element. Altho the amount of calcium in the body is many times as great as that of magnesium, most of the concentrated foods available carry very little calcium, and much more of magnesium.

* Reprint of Bulletin No. 468, December, 1919.

The functions of calcium are very important, and some of them are directly antagonistic to those of magnesium, the nearest to calcium chemically of the recognized elements of the body and of ordinary foods. But the two elements are associated in nature in many ways, and magnesium, seemingly, might serve to some extent in place of calcium for such purposes as egg-shell material when calcium is lacking. No instance was found where magnesium did this to any significant extent, or replaced calcium in the bones from which it was withdrawn for shell material, altho both elements are normal constituents of these structures.

Strontium, however, altho not a recognized constituent of the body nor of ordinary foods, was found capable of replacing calcium to a considerable extent in the egg shell and in bones as well as of accompanying or replacing calcium in other parts of the body.

Whenever rations deficient in calcium but carrying abundant supplies of magnesium were fed to the common fowl and the duck, there soon followed a noticeable shortage of calcium and of total mineral matter in the bones. With mature birds, whenever calcium was withdrawn from the skeleton it was usually taken in larger proportion from the softer bones.

When strontium salts were fed with low calcium rations for several weeks or months to mature fowls, these birds always had heavier bones with more mineral matter in them than did similar birds fed corresponding calcium or magnesium salts.

Where strontium replaced calcium in the bones of mature birds, the ratio of replacement in every instance was higher in the softer bones.

During many periods in these feeding trials, it was evident that the larger part of the material used for egg-shell construction came from the bones; in other periods, part came from the bones and part directly and indirectly from the mineral salts added to the ration; and during still other periods, the greater part of the material came directly and indirectly from the minerals fed.

Under the unusual rations, of necessity largely used in such a study, the common fowl, on the whole, showed greater tolerance for the feeding of strontium salts than did the duck. On the other hand, the duck in these feeding trials showed greater tolerance for the excess of magnesium in rations of low calcium content than did the common fowl.

The ability to save or to increase the stores of fat in the body under such rations appeared to be considerably different for the two representative species.

INTRODUCTION.

Of the mineral elements serving in the bodies of all farm animals, calcium is found in largest amount. From the feeding standpoint, also, it may be more important to consider than most other ash constituents because, under modern conditions, many rations fail to supply enough of this element.

The essential character of certain of the mineral nutrients has long been recognized, but for a time, chief attention has been given to the general energy and protein requirements of animals as supplied by the organic constituents. Of late, much has been added to our knowledge of the important rôles of the mineral elements, and, recently, especial emphasis has been put by a few leading investigators upon the importance of taking into account the calcium content of food for both man and other animals. Because of this greater consideration now given to the rôles of calcium, it seems that a few data secured in experiments relating to the study of calcium metabolism may be of enough interest to justify present publication, althò much intended work has not been done.

MAGNESIUM NOT A SUBSTITUTE FOR CALCIUM.

Besides the special duties of the base-forming elements in the body, they all serve their part in maintaining neutrality wherever necessary. In this direction, magnesium, which is nearest to calcium chemically of any recognized element in the body or its food, might serve to work with calcium to a certain extent, altho in some important functions their action is antagonistic. But in the bony framework, in so far as it serves for mechanical support, and in the shells of birds' eggs, both structures having the two elements as normal constituents, it would appear that magnesium might serve to a limited degree in place of calcium. That it does this to an extent that can be considered important or more than incidental or accidental, we have failed to find.

On the other hand, strontium, nearer to calcium chemically than other elements, can replace calcium to a certain extent, altho it is not a recognized normal constitutent of the body nor of ordinary foods.

EARLY EXPERIMENTS.

Among the first experiments undertaken at this Station, after the work with poultry began, were some to determine the availability as food of calcium in inorganic forms, particularly the carbonate. Altho oyster shell and bone were often fed by poultrymen, the use of such accessories was not general. Some influential poultrymen and writers at that time contended that calcium from such "inorganic" forms was not utilized, and that the only service rendered by such material was in a mechanical way as grit; and also some teachers in agricultural lines, who had not studied this particular matter, held the opinion that carbonate of lime as in oyster shell was unavailable.

It seemed to be the general assumption that enough calcium for other animals was always present in ordinary rations. The unusual demands for lime by the hen, however, forced attention to the insufficient supply in rations largely of grain, and it was often attempted and recommended to use too large amounts of bulky foods, otherwise inefficient, with the idea of making up this deficiency.

Altho a consideration of the low calcium content of most foods and of the large amount in the egg shell suggested a wide margin covered in some way, it seemed necessary to get more direct evidence. The reservoir of calcium in the body was not carefully considered at first or thought of much influence, as it could hardly serve for long periods unreplenished. Several feeding trials with the same object, altho somewhat different in plan, were started, but, after one series gave such pronounced results that corroboration from different angles seemed unnecessary, most of them were dropped. In the feeding trial, results from which were published, it was found during one period of careful control that over 88 percent of the calcium in the egg shells was unaccounted for by the calcium in the ordinary food and water, and no other conclusion seemed possible than that the larger part of this came from the oyster shell which carried a large excess.

Altho supplementary feeding trials were either dropped or postponed for a time, when this one immediate question seemed answered, results from a few, bearing upon the interrelation of certain elements, were so suggestive that work on this series was resumed and continued as opportunity permitted. Because it was not possible at

any one time to give sufficient attention to this line of work, the different feeding trials were scattered over a number of years at irregular intervals.

GENERAL PLAN OF EXPERIMENTS.

When first attempting to trace the transference of lime from the food to the egg shell, it seemed that, if the relative proportion deposited in the shell should vary markedly and in the same direction as in different sources of supply, there would be presumptive evidence that the material came directly or indirectly from the fluctuating source. There is a small but reasonably constant percentage of magnesium in the shell. It was a question whether this base-forming element would replace calcium to any extent when salts of magnesium were supplied with food deficient in calcium.

Because strontium is seldom found in any appreciable amount in food and ordinary water, because it approaches calcium in chemical properties, and because the ordinary salts of strontium, unlike those of barium, are not poisonous, this element seemed especially adapted for use in such an investigation. Strontium salts were, therefore, used in certain rations.

While strontium proved serviceable for such a reagent, its use was not as simple and convenient as expected. In analysis, the usual methods for estimation of strontium did not work as directly and rapidly on residues from organic material with strontium in small amounts in the presence of many other elements and the large excess of phosphorus as with simpler mineral substances. Inconveniences in analysis were finally overcome to a large extent, however.

At first several attempts were made to provide a basal ration largely of food substances so purified as to contain either little or practically none of certain ash constituents, the amount desired being supplied as added salts. But no satisfactory results were secured with any ration that did not contain considerable food in its natural, or not specially purified, state. Several rations composed largely of prepared foods served for maintenance for quite long periods without apparent harm to mature birds, but would not sustain or induce reproduction. There was no opportunity to prepare and try out any considerable assortment of the possible purified products, and there were failures to secure eggs either at all or in significant numbers from rations containing a desired

proportion of the products available. So it was necessary to use "compromise" rations with as small a proportion of the needed natural foods as would assure a fair growth of young, and eggs in significant numbers from the mature birds. There was in every basal ration an inadequate supply of calcium, however, and, as is the case with practically all rations consisting largely of grain and meat foods that do not include bone, from three to four times as much magnesium as calcium.

SELECTIVE MATERIAL IN THE EGG SHELL.

Many samples from some of the earlier feeding trials were lost before enough analytical work could be done on them, and the results are not here considered. In one trial, however, (1897) there was a failure of magnesium to replace calcium appreciably in the egg shell under conditions where strontium did replace calcium to a large extent. In this trial, four lots of hens were fed an ordinary ration, somewhat low in calcium content as usual, and were in addition supplied, in the form of ordinary grit, one lot with oyster shell, another with magnesite, another with strontianite, and a fourth with hard glass.

The percentage of calcium in the egg shells remained very constant for each lot except the lot having strontianite as grit, in which case about one-fourth of the calcium was replaced by strontium. The percentage of magnesium in the egg shells for the lot fed magnesite was about the same as for the lots having oyster shell and strontianite. In the egg shells from the hens fed oyster shell ($CaCO_3$ chiefly) there was 37.9% of Ca and 0.7% of Mg. Shells from the hens fed magnesite ($MgCO_3$) contained 37.5% of Ca and 0.85% of Mg., and shells from the hens fed strontianite ($SrCO_3$) contained 28.4% of Ca, 10.0% of Sr, and 0.8% of Mg. From the hens having glass (insoluble silicate) for grit the egg shells contained 37.8% Ca and 1.0% of Mg. The percentage of phosphorus in the shells remained practically constant, being about 0.17% of P for the lot having strontianite and close to 0.19% P for the other three lots.

In a feeding trial, some years later, with young growing pullets on rations deficient in calcium and with either magnesium or calcium added as pure salts, the first eggs laid as the pullets approached maturity showed only slight differences in the composition of the shells. There was slightly less shell with a little higher percentage

of magnesium and a somewhat lower percentage of phosphorus for the pullets that had been given magnesium salts. Shells of eggs from the pullets fed magnesium phosphate and carbonate constituted 6.2 percent of the egg and contained 0.092% of P, 1.23% of Mg, and 35.75% of Ca. Shells of eggs from pullets fed calcium phosphate and carbonate constituted 6.9 percent of the egg and contained 0.109% of P, 1.07% of Mg, and 35.79% of Ca. With both lots the amount of shell on these first eggs was considerably less (from 33% to 40%), the percentage of phosphorus in the shell less, the percentage of calcium less, and the percentage of magnesium higher than for normal eggs from mature hens having a ration of good variety and practically free range.

With young hens fed rations similarly deficient in calcium in another trial, there was the same failure of magnesium to replace calcium in the egg shell. Shells of eggs from hens fed magnesium carbonate carried a slightly smaller percentage of magnesium on the average than was in shells of eggs from hens fed either ground oyster shell or strontium carbonate. But strontium did replace calcium to a large extent in the egg shell when strontium carbonate was fed. The data are given further along in connection with other results from the same feeding trial.

The same general distribution of the principal elements in the egg shell was found when three other lots of hens were fed rations deficient in calcium as usual with different salts being added as desired. With practically the same amount of shell on the egg there was slightly more magnesium and slightly less calcium with the hens fed magnesium salts (mixed carbonate and phosphate) than with the hens fed added calcium (carbonate and phosphate), the average percentages of M6 being 1.07 and 0.75, and of Ca 35.64 and 36.19. These variations were no greater, however, than sometimes occur under identical rations. But again strontium did replace calcium in the shell to a large extent, the average of determinations made in earlier stages of feeding showing 0.74% of Mg, 26.03% of Ca, and 12.72% of Sr. The average from a few eggs of thin shells after three months' feeding showed even more strontium than calcium — 21.75% of Sr against 18.93% of Ca.

In another feeding trial, eggs from ducks fed magnesium carbonate with the usual calcium-deficient ration had shells of practically the same composition as those from ducks fed calcium carbonate. Eggs

from ducks fed strontium carbonate with the same ration showed a slight replacement of calcium by strontium even on the second day of strontium feeding.

With three other lots of ducks fed a basal ration low in calcium and with either magnesium, calcium, or strontium added as mixed carbonates and phosphates, shells of eggs from those having magnesium salts contained 0.34% of Mg and 35.52% of Ca, and from those fed calcium salts 0.36% of Mg and 34.30% of Ca. Shells of eggs (altho few) from the ducks fed strontium salts contained a little less magnesium than the others and strontium in place of part of the calcium — showing 0.25% of Mg, 12.04% of Sr, and 25.51% of Ca. Shells of eggs from ducks of the same breeding having a "normal" ration and free range showed 0.31% of Mg and 37.09% of Ca.

CALCIUM REPLACEMENT IN THE BONES.

If calcium is used in any considerable amount above that in the food and water for essential physiological functions where no substitute will serve and for such structures as the egg shell, it must come from what is stored in the body. The greater part of this reservoir of calcium is in the bones. From eight to nine percent of the total dry matter of the body of an average hen is represented by the ash constituents. A large part of this ash is calcium phosphate.

In the general study of this question, the bones were roughly grouped into two classes, the harder bones of the extremities, with the wish-bone, coracoid bones, etc., in one class, and the bones of the trunk, including the skull, etc., in the other class. It was assumed that if calcium were withdrawn from the bones it would be taken first and perhaps to a larger extent from the softer bones having more blood vessels. The results with the common fowl were usually in accord with this assumption when mature or nearly grown young birds were used. The rule did not always hold in the earlier stages of growth when the bones had not yet fully ossified, and when there was perhaps less difference between the two groups. Also young birds cannot be fed without disadvantage, as can the older birds, any considerable amount of finely powdered carbonate, even ground oyster shell. And such materials were of necessity used in some of these experiments.

The effect of the deficient rations on the softer bones was shown much more noticeably by their physical appearance than by the

figures from chemical analysis. Under some rations, the breast bone and bones of the pelvic arch, especially, lost all stiffness, became soft, flexible and translucent, and apparently were without much lime, altho analysis showed considerable of the mineral matter still remaining.

With the duck, young and old, a fowl which seems better able to adapt itself to an excess of magnesium when only a very low indispensable minimum of calcium is present, the relations between the two classes of bones in respect to changes in composition did not hold always as with the common fowl.

Where strontium replaced calcium in the bones, however, in every instance with both representative species, except in the earlier stages of feeding with immature birds, the ratio of strontium replacement was higher in the softer bones.

PRESENCE OF STRONTIUM IN THE BODY.

If strontium were transported freely thru the body, either in addition to calcium or partly as a substitute, we should expect to find it wherever its presence did not vitally interfere with the special functions of other elements.

The relative proportion of strontium to calcium found in the egg itself was not much different from that existing in the shell, and this proportion was about the same in the white as in the yolk. Usually, but not always, both in the white and in the yolk, there was less than the usual amount of calcium whenever strontium was present. The absolute amounts of these elements in the egg are small, however, and not much importance can be attached to variations in the relative amounts in the absence of sufficient data to show what may be an ordinary range of the variation in composition that has been observed under normal rations.

Strontium was found in the muscles in smaller amounts in relation to calcium than in the egg, altho the proportion was higher in the "red" muscles than in the "white" muscles.

Except in a few egg shells, the only place where strontium was found in considerably greater amount than calcium was in the smaller feathers from a hen that had been fed strontium salts for more than a year, having been carried thru a full molt. In these smaller feathers there was much more strontium than calcium,

altho in the larger feathers there was a little more of calcium than of strontium. A difference of the same kind was found in feathers from a yearling duck fed strontium salts, after reaching full size, for a period of six months not covering any period of general molt. (Under the restricted ration, any small feathers shed were eaten.) In this instance there was found half as much strontium as calcium in the smaller feathers, and about one-fifth as much in the larger feathers.

GENERAL DIFFERENCES IN BONE MATERIAL.

Whenever the old or young of the common fowl or duck were fed rations low in calcium content with added magnesium salts, there was soon found in the bones less calcium and less total mineral matter than in bones from birds fed the same ration with added calcium salts.

Usually, but not always, those birds fed rations with added magnesium salts had more magnesium in the bones than the birds fed calcium salts, altho the actual difference in amount of magnesium was always small. Except with young ducks or very immature chicks, birds fed strontium salts for any considerable time had more magnesium in the bones than those fed magnesium or calcium salts for the same time.

When strontium salts were fed for several weeks or several months with these low calcium rations, mature hens or nearly mature chicks always had heavier bones with more mineral matter in them, actually and in relation to body weight, than did similar birds fed corresponding calcium or magnesium salts.

There was a similar result when mature ducks were fed such rations for a limited time, but not with immature ducks or young ducklings or very immature chicks.

TOLERANCE FOR THE UNUSUAL RATIONS.

Under these unusual, and somewhat artificial, rations the common fowl, on the whole, was much better able to endure the feeding of strontium salts than was the duck. With variety provided in the food and no extreme deficiency of calcium, mature hens could be fed the added strontium for a long time without any noticeable injury that could be attributed to the presence of this element in the food./

Mature ducks of large size did not suffer any apparent harm during limited periods of feeding from the presence of strontium in the food. But under such rations as were used, immature ducks or growing ducklings had little tolerance for strontium carbonate or phosphate, never being in the best condition and making very slow growth. On the other hand, altho there was a shortage of calcium in the bones and a failure of magnesium to replace it, rations with added magnesium salts were practically equal in efficiency to those with added calcium salts and permitted as rapid and, except as to bone development, apparently as normal growth. The small amount of calcium in the food, supplemented by any temporarily withdrawn from the bones, apparently provided the indispensable minimum, and there seemed almost perfect tolerance for the added magnesium salts, so that in general appearance and condition of plumage the young ducks often surpassed those fed calcium salts.

With the ducklings started on these rations at the earlier age and fed for the longer periods, there was, on the average for the whole time, no difference in the amount of dry matter of the food required per pound increase in weight between those fed added magnesium and those fed added calcium. For the common chick, up to the time when nearly half grown, the ration with added magnesium permitted fully as rapid growth from the same amount of dry matter in the food as the ration with added calcium, but from this stage of growth on the efficiency of the rations with added calcium salts was increasingly the greater.

FAT IN THE BODY.

The ability to save or to increase the stores of fat in the body under these unusual rations, so far as indicated by ordinary dissection and, in several instances, by analyses of the bones, appeared to be considerably different for the two representative species.

For the common fowl there was in every trial, after several months feeding, more fat next the skin and about the body with birds fed the added calcium salts. Between those fed the magnesium or strontium salts there was not much difference on the average. The feeding of strontium salts with a varied but low-calcium ration for over a year to one hen did not prevent an increase in this proportion of stored fat. Young pullets approaching laying maturity, after they had been under these rations for six to eight months, showed a larger percentage of fat in the trunk bones from those

fed the added magnesium than from the others, and there was considerable shortage of fat in the harder bones from those having had strontium salts. Elsewhere in the body, however, there was more fat with the lot fed added calcium salts than with the other ots, between which there was little difference.

The duck, young or old, when fed the ration with added strontium salts for any considerable time showed a noticeable lack of fat in the bones and in the rest of the body. A partial exception was in the case of a laying duck fed for six months on the restricted ration when the hard bones showed no apparent loss of fat, altho there was a noticeable shortage in the softer bones and elsewhere in the body. There were abundant stores of fat in the bones and elsewhere with birds fed the added magnesium and calcium salts. On the whole, for the longer periods of feeding with the ducks, slightly more fat was held or accumulated under the rations with added magnesium than under the rations with added calcium salts.

EFFECTS ON EGG PRODUCTION.

In the matter of influence on egg production, the results, on the whole, plainly favored the rations with the larger proportion of calcium. Under the unusual rations free laying could not be expected, but three and four times as many eggs were obtained from hens fed the added calcium as from other lots. Fewer eggs were obtained from the hens fed added magnesium salts than from the hens fed strontium salts. With the younger hens, from which readier egg production would normally be expected, laying started earlier and continued longer with the lot fed added calcium salts.

Few eggs were obtained from ducks under such rations, but on the average there were a few more from those fed rations with the added calcium, altho in one trial as many eggs were obtained from the ducks fed added magnesium salts. Only very few eggs were ever obtained from ducks fed strontium salts for any considerable time.

SEPARATE FEEDING TRIALS.

YOUNG HENS.

LOTS I, II, AND III.

Special contrasted feeding started with the birds in these lots before they were mature enough to lay, and was continued for about six and one-half months. There were six in each lot at the start. After eight weeks feeding, two from each lot were removed for dissection.

They were fed cracked corn, rice, cornmeal, beef fat, apples (without seeds, etc.), meat meal (free from bone), and a mixture of corn meal, starch, blood meal, wheat flour, sugar, and cream gluten meal with a little salt. White quartz glass sand covered the wooden floor of each pen. The water used was rain water.

The calcium in the basal ration ran from about .013% to .015% of the total dry matter. There was, on the average, over three times as much magnesium, the ratio of total calcium to total magnesium averaging for the whole time about 1:3.7 for each lot.*

To the basal ration for one lot magnesium carbonate was added, to that of another strontium carbonate and to that of the third calcium carbonate. Ground magnesite was fed for the first half of the time to the extent of about 1.3% of the total dry matter of the food, and precipitated magnesium carbonate to the extent of about 2.4% of the total dry matter of the food for the rest of the time. Ground strontianite was fed for the first half of the feeding period to the extent of about 1.4% of the total dry matter of the food, and for the rest of the time precipitated strontium carbonate to the extent of about 2.4% of the total dry matter. For the calcium carbonate, oyster shell was used thruout for the first half of the time to the extent of about 1.3% of the total dry matter of the food, and of about 4% of the total dry matter for the last three months. These figures indicate only the average proportions used for the

* It was not possible to make special complete analyses of all foods used, and, for the standard foods, figures from analyses reported by Forbes, Sherman, and others have been used in computing general mineral content of the rations. Variations in the composition of these standard materials would not be great enough to affect the estimated totals to any appreciable extent, and could not effect any change in relation.

first and last halves of the feeding trial. After the first few weeks of feeding, the proportion of these added minerals in the food increased continually to the end.

Shells of the first few eggs laid by the lot (I) fed added magnesite contained fifteen times as much calcium as was in the food for the period during which these eggs were obtained. Toward the latter part of the feeding trial fifteen eggs, slowly produced by this lot, had in the shells a little over four times as much calcium as was in the food for the same period.

Shells of the first few eggs laid by the lot (II) fed strontium carbonate contained over eight times as much calcium as was in the food for the same period. Shells of twenty-three eggs slowly produced toward the end of the feeding trial contained over five times as much calcium as was in the food during the same period.

TABLE I.— SHELLS OF EGGS FROM YOUNG HENS, LOTS I, II, AND III.

Ration	SAMPLE.	Per ct. of egg as shell.	Per ct. of Ca in dry shell.	Per ct. of Sr in dry shell.	Per ct. of Mg in dry shell.	Per ct. of P in dry shell.
Lot I Magnesium carbonate	Average for 4 eggs after about 2 months feeding	7.3	35.16	.00	.82	.15
	Average for 6 eggs after about 3 months feeding	6.6	36.25	.00	.71	.16
	Average for 3 eggs after about 5 months feeding	4.7	35.82	.00	1.03	.14
Lot II Strontium carbonate	Average for 4 eggs after about 2 months feeding	7.4	28.36	9.94	.84	.18
	Average for 8 eggs after about 3 months feeding	5.6	26.75	10.05	1.10	.15
	Average for 6 eggs after about 3½ months feeding	6.2	26.58	9.94	1.09	.16
	Average for 7 eggs after about 5 months feeding	4.9	22.90	15.39	.92	.18
Lot III Calcium carbonate	Average for 4 eggs after about 3 months feeding	7.9	35.81	.00	.84	.13
	Average for 9 eggs after about 3 months feeding	5.8	35.96	.00	.84	.12
	Average for 6 eggs after about 3½ months feeding	6.3	35.91	.00	.86	.16
	Average for 8 eggs after about 5 months feeding	6.4	35.99	.00	1.15	.16

TABLE II.— YOUNG HENS.

	LOT I MAGNESIUM CARBONATE.		LOT II STRONTIUM CARBONATE.		LOT III CALCIUM CARBONATE.	
	Average of two birds after eight weeks feeding.	Average of three birds after 6½ months feeding.	Average of two birds after eight weeks feeding.	Average of three birds after 6½ months feeding.	Average of two birds after eight weeks feeding.	Average of two birds after 6½ months feeding.
Percent of weight of body* represented by dry bones	7.5	7.1	8.9	8.3	7.0	7.0
Percent of dry matter in fresh bones	64.1	63.9	69.7	62.4	61.8	56.3
Percent of dry matter in fresh bones of extremities	65.9	69.7	71.1	65.9	63.8	60.0
Percent of dry matter in fresh bones of trunk	61.1	56.3	67.5	59.0	58.8	53.1
Percent of weight of body* represented by ash of bones	3.02	2.62	3.98	3.69	3.00	2.96
Percent of ash in dry bones	40.4	36.8	44.9	44.4	42.7	42.1
Percent of ash in dry bones of extremities	42.5	38.7	45.0	47.0	45.1	47.1
Percent of ash in dry bones of trunk	37.2	33.4	44.6	39.7	38.8	34.8

* The "weight of body" used for the comparisons given in all the tables in this bulletin is the weight after removal of the feathers and of the organs of the abdominal cavity.

TABLE III.— YOUNG HENS.

	Lot I Magnesium Carbonate.		Lot II Strontium Carbonate.		Lot III Calcium Carbonate.		Average at start of feeding.
	Average of two birds after eight weeks feeding.	Average of three birds after 6½ months feeding.	Average of two birds after eight weeks feeding.	Average of three birds after 6½ months feeding.	Average of two birds after eight weeks feeding.	Average of two birds after 6½ months feeding.	
Percent of Ca in ash of bones of extremities	38.15	37.90	33.66	33.54	37.88	37.76	37.97
Percent of Sr in ash of bones of extremities	.00	.00	4.24	3.74	.00	.00	.00
Percent of Mg in ash of bones of extremities	.77	.86	1.01	1.48	.90	.70	1.23
Percent of P in ash of bones of extremities	17.66	17.57	17.55	16.78	17.48	17.16	17.66
Percent of Ca in ash of bones of trunk	37.80	36.49	33.13	31.00	37.89	37.33	37.59
Percent of Sr in ash of bones of trunk	.00	.00	5.39	5.01	.00	.00	.00
Percent of Mg in ash of bones of trunk	.80	.93	1.07	1.52	.90	.71	1.05
Percent of P in ash of bones of trunk	17.54	17.50	17.50	16.80	17.60	17.27	17.57
Ca in bones of extremities, grams	6.75	5.57	8.18	6.45	7.42	6.74	....
Sr in bones of extremities, grams	.00	.00	1.03	.72	.00	.00	....
Mg in bones of extremities, grams	.14	.13	.25	.28	.18	.12	....
P in bones of extremities, grams	3.13	2.58	4.26	3.23	3.43	3.05	....
Ca in bones of trunk, grams	3.67	2.68	4.84	3.22	4.02	3.37	....
Sr in bones of trunk, grams	.00	.00	.79	.52	.00	.00	....
Mg in bones of trunk, grams	.08	.07	.16	.16	.10	.06	....
P in bones of trunk, grams	1.70	1.29	2.56	1.75	1.87	1.56	....

Total Ca in bones, grams	10.42	8.25	13.02	9.67	11.44	10.11	....
Total Sr in bones, grams	.00	.00	1.82	1.24	.00	.00	....
Total Mg in bones, grams	.22	.20	.41	.44	.28	.18	....
Total P in bones, grams	4.83	3.87	6.82	4.98	5.30	4.61	....
Percent of dry bones as Ca	15.37	13.75	15.02	14.48	16.16	15.84	....
Percent of dry bones as Sr	.00	.00	2.10	1.85	.00	.00	....
Percent of dry bones as Mg	.32	.33	.47	.66	.39	.28	....
Percent of dry bones as P	7.12	6.45	7.87	7.46	7.63	7.23	....
Percent of weight of body represented by Ca in bones	1.15	.98	1.33	1.20	1.14	1.11	....
Percent of weight of body represented by Ca and Sr in bones	....	....	1.52	1.36	....	....	....
Percent of weight of body represented by Mg in bones	.024	.024	.042	.055	.028	.020	....
Percent of weight of body represented by P in bones	.53	.46	.70	.62	.53	.51	....

For the lot (III) fed added calcium carbonate, the ratio of the total calcium in all other food to the calcium in the shells of the eggs produced during the time the food was eaten was: For the first 26 eggs produced about 1:20.5, for the last 26 eggs produced about 1:60.1, and for most of the eggs laid in the intervening period about 1:26.7. For a total of six months under this ration the calcium in all the food aside from the added oyster shell was only 4.2% of the amount in the shells of eggs laid during the same period.

The larger part of the calcium used as egg material during this feeding trial evidently came, sometimes from the bones and sometimes directly and indirectly from the oyster shell fed. Certain results of analyses of many of the eggs from these hens are shown by table I, and some of the data obtained by examination of the bones of most of the birds from lots I, II, and III are given in accompanying tables II and III.

CHICKS.

LOTS IV, V, AND VI.

With these three lots of chicks, special contrasted feeding started when they had reached the average live weight of a little less than a pound. There were seven birds in each lot at the start. Three of each lot were fed for six to seven months; the others were taken out for examination and dissection after different intervals, mostly after thirteen and eighteen weeks under these rations.

They were fed rice, sifted cornmeal, beef fat, apples (without seeds etc.), a little blood meal and meat meal (free from bone), and a mixture of wheat flour, rice flour, sifted corn meal, starch, sugar, and wheat gluten. They had rain water, containing but little mineral matter, for drink. The amount of calcium in the basal ration was small, running from .013% to .014% of the total dry matter. There was nearly four times as much magnesium, the ratio of total calcium to total magnesium for most of the time being from 1:3.8 to 1:3.9.

To the basal ration for one lot (IV) there was added a mixture of four parts of magnesium phosphate and one part of magnesium carbonate to the extent of from 1.6% to 1.7% of the total dry matter of the food. Another lot (V) was fed a mixture of four parts of strontium phosphate and one part of strontium carbonate, constituting at different times from 1.6% to 1.8% of the total dry matter of the food. There was fed to another lot a mixture of four parts

TABLE IV.— CHICKS.

	LOT IV MAGNESIUM PHOSPHATE AND CARBONATE.		LOT V STRONTIUM PHOSPHATE AND CARBONATE.		LOT VI CALCIUM PHOSPHATE AND CARBONATE.		Average type at beginning.
	Average of two birds after 18 weeks feeding.	Average of three birds after 6 to 7 months feeding.	Average of three birds after 6 to 17 weeks feeding.	Average of three birds after 6 to 7 months feeding.	Average of two birds after 18 weeks feeding.	Average of three birds after 6 to 7 months feeding.	
Percent of weight of body represented by dry bones	7.3	7.2	8.9	8.1	8.8	7.2	11.3
Percent of dry matter in fresh bones	56.3	56.8	49.3	53.3	64.1	62.1	55.1
Percent of dry matter in fresh bones of extremities	59.2	60.6	50.2	55.2	69.6	64.7	57.3
Percent of dry matter in fresh bones of trunk	52.2	51.8	47.9	50.5	57.6	59.1	51.6
Percent of weight of body represented by ash of bones	3.23	2.93	2.78	3.22	4.13	3.19	....
Percent of ash in dry bones	44.1	40.7	31.1	39.7	46.8	44.1	....
Percent of ash in dry bones of extremities	47.1	44.7	31.5	44.1	47.1	47.0	....
Percent of ash in dry bones of trunk	39.2	34.4	30.5	32.7	46.3	39.5	....
Percent of fat in dry bones of extremities	....	11.8	....	7.6	....	11.7	....
Percent of fat in dry bones of trunk	....	17.5	....	14.0	....	14.0	....

TABLE V.— CHICKS.

	LOT IV MAGNESIUM PHOSPHATE AND CARBONATE.		LOT V STRONTIUM PHOSPHATE AND CARBONATE.		LOT VI CALCIUM PHOSPHATE AND CARBONATE.	
	Average of two birds after 18 weeks feeding.	Average of three birds after 6 to 7 months feeding.	Average of three birds after 16 to 17 weeks feeding.	Average of three birds after 6 to 7 months feeding.	Average of two birds after 18 weeks feeding.	Average of three birds after 6 to 7 months feeding.
Percent of Ca in ash of bones of extremities	35.90	36.87	25.37	30.08	37.50	36.98
Percent of Sr in ash of bones of extremities	.00	.00	9.13	9.39	.00	.00
Percent of Mg in ash of bones of extremities	1.13	.80	.70	1.08	.67	.56
Percent of P in ash of bones of extremities	18.16	18.26	17.48	18.20	18.26	17.96
Percent of Ca in ash of bones of trunk	36.44	36.04	27.53	29.01	36.99	37.19
Percent of Sr in ash of bones of trunk	.00	.00	7.51	9.30	.00	.00
Percent of Mg in ash of bones of trunk	1.18	.79	1.24	.78	.67	.59
Percent of P in ash of bones of trunk	18.16	17.92	17.08	18.20	17.60	17.88
Ca in bones of extremities, grams	6.06	7.12	2.79	5.81	7.13	8.95
Sr in bones of extremities, grams	.00	.00	1.00	1.81	.00	.00
Mg in bones of extremities, grams	.19	.15	.08	.21	.13	.14
P in bones of extremities, grams	3.07	3.52	1.92	3.51	3.47	4.35
Ca in bones of trunk, grams	3.19	3.53	1.75	2.61	4.86	5.32
Sr in bones of trunk, grams	.00	.00	.48	.84	.00	.00
Mg in bones of trunk, grams	.10	.08	.09	.07	.09	.08
P in bones of trunk, grams	1.59	1.76	1.08	1.64	2.31	2.56

Total Ca in bones, grams	9.25	10.65	4.54	8.42	11.99	14.27
Total Sr in bones, grams	.00	.00	1.48	2.65	.00	.00
Total Mg in bones, grams	.29	.23	.17	.28	.22	.22
Total P in bones, grams	4.66	5.28	3.00	5.15	5.78	6.91
Percent of dry bones as Ca	15.89	14.90	8.15	11.81	17.43	16.27
Percent of dry bones as Sr	.00	.00	2.66	3.72	.00	.00
Percent of dry bones as Mg	.50	.32	.31	.39	.32	.25
Percent of dry bones as P	8.01	7.38	5.39	7.22	8.40	7.88
Percent of weight of body represented by Ca in bones	1.17	1.07	.73	.96	1.54	1.18
Percent of weight of body represented by Ca and Sr in bones	......	....	.98	1.26	....	....
Percent of weight of body represented by Mg in bones	.037	.023	.027	.032	.028	.018
Percent of weight of body represented by P in bones	.59	.53	.48	.59	.74	.57

of calcium phosphate and one part of calcium carbonate, the proportion in the ration varying but little from that of 1.7% of the total dry matter of the food. Tribasic phosphates were used in these mixtures.

On the whole, growth was slowest and made at a somewhat greater expenditure of food by the lot fed strontium salts, and was fastest and at a little less expenditure of food by the lot fed calcium salts. But for the first half of the time, under these rations, the lot fed magnesium salts made somewhat the fastest growth and at considerably less expenditure of food.

Some of the results of dissection and analyses of the bones from most of the birds used in this feeding trial are given in tables IV and V.

MATURE HENS.

LOTS VII, VIII, AND IX.

After general preparatory feeding, these three lots of hens were fed special restricted rations for about four months, altho one bird was removed from each pen for dissection before the full period of feeding. There were three hens about one year old in each lot at the start. One hen of lot VIII was kept under the special ration for over a year.

All were fed rice, sifted corn meal, beef fat, blood meal, meat meal (free from bone), apples (without seeds etc.), and a mixture of rice flour, sifted corn meal, starch, wheat flour, sugar, and wheat gluten. After the first six weeks a little egg (without shell) was also fed, and for the last two weeks sour milk whey. The hen fed for over a year had the egg for part of the time during the first six months of the year and sour milk whey during the last month of the year's feeding. Rain water was used for drink, and floors of the pens were covered with white quartz glass sand.

The percentage of total calcium in the basal ration ran from .012 to .03 and averaged about .02% of the total dry matter. There was always more magnesium than calcium, altho the ratio varied considerably at different times. On the average for the whole time, there was more than twice as much magnesium as calcium.

To the basal ration for one lot (VII), a mixture of magnesium phosphate and magnesium carbonate was added, to that of another (VIII) strontium phosphate and carbonate, and to that of a third

(IX) calcium phosphate and carbonate. For the first six weeks these added salts consisted of equal parts of phosphate and carbonate, but for the rest of the time twice as much of carbonate as of phosphate was fed. For the hen fed strontium salts for over a year the relative amounts of carbonate and phosphate varied considerably at different times, but for most of the time more carbonate than phosphate was fed. On the average, for the whole period of feeding, the proportion of these added salts in the food was nearly the same for each lot, being in amount equal to about 1.8% of the total dry matter of the ration, but the variations in the proportions that could be fed satisfactorily were not alike. With the magnesium salts this variation was from about 1.3 to 2.6% of the total dry matter in the food, with the strontium salts from about 1.7 to 2.2%, and with the calcium salts from about 1.4 to 2.4% of the total dry matter. The smaller proportions were fed in every case for the first few weeks and the larger proportions for the last few weeks of the entire period of feeding.

TABLE VI.—SHELLS OF EGGS FROM HENS, LOTS VII, VIII, AND IX.

Ration	Sample	Per ct. of egg as shell	Per ct. of Ca in dry shell	Per ct. of Sr in dry shell	Per ct. of Mg in dry shell	Per ct. of P in dry shell
Lot VII Magnesium Carbonate and Phosphate	Average for 6 eggs after about 1 months feeding..........	6.4	36.22	.00	.91	.12
	Average for 3 eggs after about 2½ months feeding.........	6.2	35.00	.00	......	.18
Lot VIII Strontium Carbonate and Phosphate	Average for 3 eggs after about 1 weeks feeding...........	6.1	33.13	3.69	.81	.....
	Average for 8 eggs after about 3 months feeding..........	4.0	18.93	21.75	.66	.14
	Average for 7 eggs after about 1 year's feeding...........	4.1	22.07	18.71	......	.....
Lot IX Calcium Carbonate and Phosphate	Average for 12 eggs after about 3 months feeding..........	6.7	36.34	.00	.74	.17

TABLE VII.—MATURE HENS.

	LOT VII MAGNESIUM CARBONATE AND PHOSPHATE	LOT VIII STRONTIUM CARBONATE AND PHOSPHATE	LOT IX CALCIUM CARBONATE AND PHOSPHATE	LOT VIII STRONTIUM CARBONATE AND PHOSPHATE
	Average of three birds after 11 to 18 weeks feeding.	Average of two birds after 11 to 18 weeks feeding	Average of two birds after 6 to 18 weeks feeding.	One hen after more than one year's feeding.
Percent of weight of body represented by dry bones..	9.5	9.7	10.0	8.6
Percent of dry matter in fresh bones............	50.6	62.6	60.9	53.9
Percent of dry matter in fresh bones of extremities.	54.4	67.3	64.5	58.7
Percent of dry matter in fresh bones of trunk......	46.9	56.8	56.5	48.6
Percent of weight of body represented by ash of bones	3.81	4.29	3.98	3.48
Percent of ash in dry bones...	40.0	44.0	39.6	40.1
Percent of ash in dry bones of extremities...........	43.2	45.8	44.9	47.0
Percent of ash in dry bones of trunk...............	35.5	41.5	36.4	32.1

During one period of six weeks the total calcium in the food for Lot VII, fed magnesium salts, was less than 7% of the amount of calcium in the shells of eggs laid during that time. For a short period with the lot fed strontium salts there was in the food about 5.2% as much calcium as was in the shells of eggs produced. With Lot IX, fed calcium salts, there was in the basal ration for one period of six weeks about 5.6% as much calcium as was in the shells of the eggs laid during this period. During two other periods, one of five and one of four weeks, there was in the food, aside from the added calcium salts in each period, less than 4% of the amount of calcium in the shells of eggs produced during the same period. In some of these instances the larger part of the calcium used in constructing the egg shell came directly and indirectly from the calcium salts fed. In one instance the larger part of this calcium required came from the bones.

During one period of about three weeks with Lot VIII fed strontium salts the shells of the eggs produced carried three and one-half times as much calcium as was in the food, and about fifteen percent more of strontium than of calcium, no strontium being in the basal ration. In this instance the larger part of the calcium in the eggs came from the bones, and the strontium directly and indirectly from the mineral salts fed.

In table VI are shown some of the results of analyses of a number of eggs from each of these lots, and in tables VII and VIII some of the data from analyses of bones from the hens of lots VII, VIII, and IX.

TABLE VIII.— MATURE HENS.

	LOT VII MAGNESIUM CARBONATE AND PHOSPHATE	LOT VIII STRONTIUM CARBONATE AND PHOSPHATE	LOT IX CALCIUM CARBONATE AND PHOSPHATE	LOT VIII STRONTIUM CARBONATE AND PHOSPHATE
	Average of three birds after 11 to 18 weeks feeding.	Average of two birds after 11 to 18 weeks feeding.	Average of two birds after 6 to 18 weeks feeding.	One hen after more than one year's feeding.
Percent of Ca in ash of bones of extremities	37.18	35.45	37.62	34.66
Percent of Sr in ash of bones of extremities	.00	4.06	.00	4.75
Percent of Mg in ash of bones of extremities	.82	.62	.59	1.14
Percent of P in ash of bones of extremities	17.76	17.60	18.40	16.99
Percent of Ca in ash of bones of trunk	35.93	33.59	36.87	31.94
Percent of Sr in ash of bones of trunk	.00	6.42	.00	6.57
Percent of Mg in ash of bones of trunk	.98	.71	.66	1.19
Percent of P in ash of bones of trunk	17.64	17.60	17.62	16.92
Ca in bones of extremities, grams	5.31	7.07	6.37	7.01
Sr in bones of extremities, grams	.00	.81	.00	.96

TABLE VIII (*continued*).

	LOT VII MAGNESIUM CARBONATE AND PHOSPHATE	LOT VIII STRONTIUM CARBONATE AND PHOSPHATE	LOT IX CALCIUM CARBONATE AND PHOSPHATE	LOT VIII STRONTIUM CARBONATE AND PHOSPHATE
	Average of three birds after 11 to 18 weeks feeding.	Average of two birds after 11 to 18 weeks feeding.	Average of two birds after 6 to 18 weeks feeding.	One hen after more than one year's feeding.
Mg in bones of extremities, grams	.12	.12	.10	.23
P in bones of extremities, grams	2.53	3.51	3.11	3.44
Ca in bones of trunk, grams	3.02	4.13	3.33	3.30
Sr in bones of trunk, grams	.00	.79	.00	.68
Mg in bones of trunk, grams	.08	.09	.06	.12
P in bones of trunk, grams	1.48	2.16	1.59	1.75
Total Ca in bones, grams	8.33	11.20	9.70	10.31
Total Sr in bones, grams	.00	1.60	.00	1.64
Total Mg in bones, grams	.20	.21	.16	.35
Total P in bones, grams	4.01	5.67	4.70	5.19
Percent of dry bones as Ca	14.69	15.32	14.80	13.71
Percent of dry bones as Sr	.00	2.19	.00	2.18
Percent of dry bones as Mg	.35	.29	.24	.47
Percent of dry bones as P	7.07	7.76	7.18	6.90
Percent of weight of body represented by Ca in bones	1.40	1.49	1.49	1.17
Percent of weight of body represented by Ca and Sr in bones	....	1.72	....	1.36
Percent of weight of body represented by Mg in bones	.034	.028	.025	.040
Percent of weight of body represented by P in bones	.67	.75	.72	.59

YOUNG DUCKS.

LOTS I, II, AND III.

These young ducks were placed under the restricted rations when about half grown, and fed for seven weeks until growth had become very slow and full size for the type practically reached. There were

four birds in each lot for the whole period, and five in each lot for the first three weeks.

They were fed rice, corn meal, beef fat, apples (without seeds etc.), a little blood meal and meat meal (free from bone), and a mixture of corn meal, starch, blood meal, sugar, wheat flour, and cream gluten meal with a little common salt. White quartz sand was mixed in the food and covered the floor. The only water used was rain water. The total calcium in the basal ration averaged from .016% to .017% of the total dry matter, and the total magnesium ran from 3.1 to 3.3 times as much as this.

To the ration for Lot I magnesium carbonate was added to an extent that averaged for the whole period about 3.4% of the total dry matter, to that for Lot II strontium carbonate to an average extent of about 2.8% of the total dry matter, and to the ration for Lot III calcium carbonate to an average extent of 3.3% of the total dry matter. The proportion of the added carbonate in the ration was increased gradually from the start of feeding as fast as was possible without seriously affecting the palatability of the food.

Under these unusual rations growth was rapid and fairly normal for Lots I and III. The birds in Lot III on the average for the whole period made somewhat more rapid growth on a lesser amount of dry matter in the food, and attained a little larger average size, altho as high individual weight was found in Lot I. But during the first three weeks the growth was a little faster and somewhat less food was required per pound gain for Lot I. In the latter stages of feeding Lot I ate somewhat less than Lot III and more restlessness and the habit of looking for other material developed, such as pulling and picking at cloth that could be reached at one end of the run, but feather pulling did not appear. In Lot II, however, it was much trouble to control the tendency to feather pulling shown by some of the birds, and at times they persisted in attempts to get such things as nail heads showing in the floor and sides of pen. With Lot II growth was very slow and full size was not attained.

The harder bones from Lot II fed the strontium salt, when dry, were yellower and much lighter in color than those from the other lots; the bones from Lot III were lighter colored than those from Lot I. The bones from Lot II contained unusually small amounts of fat and

were tougher and harder to reduce in a mortar than corresponding bones from the other lots. The reduced sample of the harder bones, while moist from some fat, made a crumbly mass, but samples from the lots fed added calcium or magnesium carbonates, when reduced in a mortar, made an oily, pasty mass, very different in consistency.

Data obtained by analyses of the bones from a majority of the birds in these three lots are given in accompanying tables IX and X.

TABLE IX.— YOUNG DUCKS.

	LOT I MAGNESIUM CARBONATE	LOT II STRONTIUM CARBONATE	LOT III CALCIUM CARBONATE	Average at start of feeding.
	Average of three birds after seven weeks feeding.	Average of four birds after seven weeks feeding.	Average of two birds after seven weeks feeding	
Percent of weight of body represented by dry bones..	10.6	10.1	10.3	10.1
Percent of dry matter in fresh bones.............	62.6	47.4	66.4	49.1
Percent of dry matter in fresh bones of extremities.	70.8	51.3	72.7	51.2
Percent of dry matter in fresh bones of trunk......	53.7	43.3	58.8	47.0
Percent of weight of body represented by ash of bones	3.27	2.94	3.59	3.73
Percent of ash in dry bones..	30.7	29.0	34.7	36.9
Percent of ash in dry bones of extremities...........	27.2	27.2	31.3	35.3
Percent of ash in dry bones of trunk...............	35.6	31.2	39.9	38.6

TABLE X.— YOUNG DUCKS.

	LOT I MAGNESIUM CARBONATE	LOT II STRONTIUM CARBONATE	LOT III CALCIUM CARBONATE	Average at start of feeding.
	Average of three birds after seven weeks feeding.	Average of four birds after seven weeks feeding.	Average of two birds after seven weeks feeding.	
Percent of Ca in ash of bones of extremities	37.03	27.15	38.26	34.85
Percent of Sr in ash of bones of extremities	.00	8.35	.00	.00
Percent of Mg in ash of bones of extremities	1.31	1.12	.72	1.23
Percent of P in ash of bones of extremities	17.52	17.18	17.34	16.68
Percent of Ca in ash of bones of trunk	36.60	27.61	37.37	36.06
Percent of Sr in ash of bones of trunk	.00	8.83	.00	.00
Percent of Mg in ash of bones of trunk	1.32	1.18	.83	1.03
Percent of P in ash of bones of trunk	17.60	16.98	17.06	17.40
Ca in bones of extremities, grams	9.22	4.86	11.62	5.67
Sr in bones of extremities, grams	.00	1.49	.00	.00
Mg in bones of extremities, grams	.32	.20	.22	.20
P in bones of extremities, grams	4.36	3.08	5.27	2.71
Ca in bones of trunk, grams	8.31	4.64	9.56	5.67
Sr in bones of trunk, grams	.00	1.48	.00	.00
Mg in bones of trunk, grams	.30	.20	.21	.16
P in bones of trunk, grams	4.00	2.85	4.36	2.73
Total Ca in bones, grams	17.53	9.50	21.18	11.34
Total Sr in bones, grams	.00	2.97	.00	.00
Total Mg in bones, grams	.62	.40	.43	.36
Total P in bones, grams	8.36	5.93	9.63	5.44
Percent of dry bones as Ca	11.31	7.92	13.15	13.08
Percent of dry bones as Sr	.00	2.48	.00	.00
Percent of dry bones as Mg	.40	.33	.27	.42
Percent of dry bones as P	5.39	4.95	5.98	6.27

TABLE X (*continued*).

	LOT I MAGNESIUM CARBONATE	LOT II STRONTIUM CARBONATE	LOT III CALCIUM CARBONATE	Average at start of feeding.
	Average of three birds after seven weeks feeding.	Average of four birds after seven weeks feeding.	Average of two birds after seven weeks feeding.	
Percent of weight of body represented by Ca in bones..	1.20	.80	1.36	1.32
Percent of weight of body represented by Ca and Sr in bones..................	....	1.05	....	....
Percent of weight of body represented by Mg in bones..	.043	.034	.028	.042
Percent of weight of body represented by P in bones...	.57	.50	.62	.63

DUCKLINGS.

LOTS V, VI, AND VII.

These ducklings were placed under the special restricted rations when they had reached the average weight of about one pound, and were kept under them for ten weeks until growth became very slow as the birds approached maturity. Four birds in each lot were fed for the full time and six birds for the first six weeks.

They were fed rice, corn meal, beef fat, a little blood meal and meat meal (free from bone), and a mixture of corn meal, wheat flour, rice flour, starch, sugar, and wheat gluten with a little common salt. White quartz glass sand covered the floors and some was mixed with the food. Rain water was used for drink. The amount of total calcium in all the food eaten, aside from the added salts, was about .013% of the total dry matter for each lot. The amount of magnesium in the food varied slightly for the different lots, but, on the average for the whole time, there was from 3.7 to 3.9 times as much as of calcium.

To the ration for one lot (V) there was added a mixture of four parts of magnesium phosphate and one part of magnesium carbonate, to

the ration for the second lot (VI) a mixture of four parts of strontium phosphate and one part of strontium carbonate, and to the ration for the third lot (VII) a mixture of four parts of calcium phosphate and one part of calcium carbonate. The proportion of these added salts in the ration varied slightly with each lot. The amount fed at the start, practically alike for each lot, was increased gradually so far as it could be without seriously affecting the palatability of the food. The magnesium salts were fed to an extent varying from 2.0% of the total dry matter in the food for the first few weeks to about 2.6% for the last few weeks. The strontium salts represented about 1.9% of the total dry matter of the food for the first few weeks and about 2.3% for the last few weeks. The extent to which calcium salts were fed varied from about 2.1% of the total dry matter for the first few weeks to about 2.5% for the last few weeks.

TABLE XI.— DUCKLINGS.

	LOT V MAGNESIUM PHOSPHATE AND CARBONATE	LOT VI STRONTIUM PHOSPHATE AND CARBONATE	LOT VII CALCIUM PHOSPHATE AND CARBONATE
	Average of three birds after nine weeks feeding.	Average of four birds after nine weeks feeding.	Average of two birds after nine weeks feeding.
Percent of weight of body represented by dry bones	10.0	10.3	10.0
Percent of dry matter in fresh bones	65.9	50.9	69.0
Percent of dry matter in fresh bones of extremities	71.7	54.9	74.7
Percent of dry matter in fresh bones of trunk	59.2	46.2	63.0
Percent of weight of body represented by ash of bones	3.22	2.71	3.64
Percent of ash in dry bones	32.2	26.3	36.6
Percent of ash in dry bones of extremities	27.7	25.5	34.2
Percent of ash in dry bones of trunk	38.3	27.4	39.7
Percent of fat in dry bones of extremities	41.8	30.7	37.2
Percent of fat in dry bones of trunk	19.5	16.0	17.8

TABLE XII.— DUCKLINGS.

	LOT V MAGNESIUM PHOSPHATE AND CARBONATE	LOT VI STRONTIUM PHOSPHATE AND CARBONATE	LOT VII CALCIUM PHOSPHATE AND CARBONATE
	Average of three birds after nine weeks feeding.	Average of four birds after nine weeks feeding.	Average of two birds after nine weeks feeding.
Percent of Ca in ash of bones of extremities.	37.97	29.76	38.59
Percent of Sr in ash of bones of extremities.	.00	8.32	.00
Percent of Mg in ash of bones of extremities.	1.09	1.40	1.03
Percent of P in ash of bones of extremities.	17.70	17.60	17.73
Percent of Ca in ash of bones of trunk ...	37.59	26.84	37.99
Percent of Sr in ash of bones of trunk	.00	8.82	.00
Percent of Mg in ash of bones of trunk ...	1.20	1.43	.89
Percent of P in ash of bones of trunk	17.73	17.46	17.98
Ca in bones of extremities, grams........	10.14	5.25	12.30
Sr in bones of extremities, grams.........	.00	1.47	.00
Mg in bones of extremities, grams........	.29	.25	.33
P in bones of extremities, grams..........	4.73	3.11	5.65
Ca in bones of trunk, grams.............	10.20	3.60	10.64
Sr in bones of trunk, grams..............	.00	1.18	.00
Mg in bones of trunk, grams.............	.33	.19	.25
P in bones of trunk, grams..............	4.81	2.34	5.03
Total Ca in bones, grams................	20.34	8.85	22.94
Total Sr in bones, grams................	.00	2.65	.00
Total Mg in bones, grams................	.62	.44	.58
Total P in bones, grams.................	9.54	5.45	10.68
Percent of dry bones as Ca..............	12.16	7.49	14.00
Percent of dry bones as Sr...............	.00	2.24	.00
Percent of dry bones as Mg..............	.37	.37	.35
Percent of dry bones as P...............	5.71	4.61	6.52
Percent of weight of body represented by Ca in bones........................	1.22	.77	1.40
Percent of weight of body represented by Ca and Sr in bones..................	....	1.00	....
Percent of weight of body represented by Mg in bones........................	.037	.038	.035
Percent of weight of body represented by P in bones..........................	.57	.47	.65

Growth made by the lot fed strontium salts, altho for the first three weeks at a good rate was on the whole slow, and the birds did not attain full size. The amount of food required for the growth made was not excessive and under the circumstances was surprisingly low, being only eleven percent higher than for the other lots. Food was used efficiently by lots V and VII. The amount of dry matter required for each pound gain in weight, on the average for the whole time, was the same for the two lots. During the first six weeks, the time of fastest growth, the increase was somewhat more rapid in the lot fed added calcium salts, and was made at a little more efficient expenditure of food. A little higher average weight was ultimately reached by the birds fed the added magnesium salts, but not enough to be important when so few birds are used.

Some of the data obtained by analyses of the bones from many of the birds from these lots are given in tables XI and XII.

DUCKS.

LOTS IX, X, AND XI.

After general preparatory feeding for two months on rations low in mineral matter, special contrasted feeding was continued with these ducks for about ten weeks until they were killed. There were two birds in Lot XI and three in each of the other lots. They were mature Pekin ducks averaging about eight pounds in weight at the beginning of the trial.

They were fed corn meal, rice, beef fat, apples (without seeds etc.), blood meal, meat meal (free from bone), and a mixture of corn meal, blood meal, starch, wheat flour, and sugar with a small amount of common salt. White quartz sand was mixed in the food and rain water was freely supplied. The amount of calcium in the basal ration averaged for the whole time about .018% of the total dry matter of the food for each lot.

To the ration for Lot IX magnesium carbonate was added to an extent that averaged about 2.7% of the total dry matter of the food. Lot X was fed strontium carbonate in about the same amount, averaging 2.7% of the total dry matter of the food, and calcium carbonate was fed to Lot XI to a similar extent of about 2.8% of the total dry matter of the food.

All the birds lost in weight, the rate of loss being heaviest during the first two weeks under these unusual rations. The loss in weight was more gradual and less in total amount with the birds fed calcium carbonate.

Very few eggs were obtained from the ducks. But, during one period, eggs laid by Lot XI had seven times as much calcium in the shells as was in all the food for the same period aside from the added calcium carbonate. Some of the results of analyses of eggs given in table XIII show the general differences in the composition of the shells of eggs from the three lots.

TABLE XIII.—SHELLS OF EGGS FROM DUCKS, LOTS IX, X, AND XI.

Ration	Sample.	Per ct. of egg as shell	Per ct. of Ca in dry shell	Per ct. of Sr in dry shell	Per ct. of Mg in dry shell	Per ct. of P in dry shell
Lot IX Magnesium Carbonate	Average from several eggs before preliminary feeding, and 2 months before restricted feeding	....	37.28	.00	.59	.21
	Average from 2 eggs after about 1 weeks feeding	6.4	35.93	.00	.46	.16
	From 1 egg after about 5 weeks feeding	6.2	35.83	.00	.53	.14
Lot X Strontium Carbonate	Average from 2 eggs after 2 days feeding	7.3	35.04	.79	.77	.17
Lot XI Calcium Carbonate	Average from 3 eggs after about 2 weeks feeding	6.2	35.12	.00	.51	.19
	From 1 egg after about 4 weeks feeding	5.4	35.96	.00	.59	.14
	Average from 2 eggs after about 5 weeks feeding	7.8	36.79	.00	.55	.20

There was much less fat in the hard bones of those birds fed the strontium carbonate than in the corresponding bones of the birds from either of the other lots. In tables XIV and XV are given some of the data obtained by analyses of the bones of several of the ducks from IX, X, and XI.

TABLE XIV.— MATURE DUCKS.

	LOT IX MAGNESIUM CARBONATE.	LOT X STRONTIUM CARBONATE.	LOT XI CALCIUM CARBONATE.
	Average of two birds after nine and ten weeks feeding.	Average of two birds after ten weeks feeding.	One bird after ten weeks feeding.
Percent of weight of body represented by dry bones	9.5	10.9	8.4
Percent of dry matter in fresh bones	73.8	76.2	71.8
Percent of dry matter in fresh bones of extremities	76.2	80.3	74.7
Percent of dry matter in fresh bones of trunk	70.9	71.0	68.4
Percent of weight of body represented by ash of bones	3.67	4.87	3.72
Percent of ash in dry bones	38.5	44.8	44.4
Percent of ash in dry bones of extremities	32.4	43.5	41.8
Percent of ash in dry bones of trunk	46.9	46.6	47.8

TABLE XV.— MATURE DUCKS.

	LOT IX MAGNESIUM CARBONATE.	LOT X STRONTIUM CARBONATE.	LOT XI CALCIUM CARBONATE.
	Average of two birds after nine and ten weeks feeding.	Average of two birds after ten weeks feeding.	One bird after ten weeks feeding.
Percent of Ca in ash of bones of extremities	38.91	37.21	38.79
Percent of Sr in ash of bones of extremities	.00	1.62	.00
Percent of Mg in ash of bones of extremities	.79	.73	.81
Percent of P in ash of bones of extremities	17.28	17.36	17.48
Percent of Ca in ash of bones of trunk	38.25	36.84	38.81
Percent of Sr in ash of bones of trunk	.00	1.91	.00
Percent of Mg in ash of bones of trunk	.69	.71	.76
Percent of P in ash of bones of trunk	17.24	17.18	17.32

TABLE XV.— (*concluded*).

	LOT IX MAGNESIUM CARBONATE.	LOT X STRONTIUM CARBONATE.	LOT XI CALCIUM CARBONATE.
	Average of two birds after nine and ten weeks feeding.	Average of two birds after ten weeks feeding.	One bird after ten weeks feeding.
Ca in bones of extremities, grams	13.54	19.28	17.12
Sr in bones of extremities, grams	.00	.84	.00
Mg in bones of extremities, grams	.27	.38	.36
P in bones of extremities, grams	6.01	9.00	7.72
Ca in bones of trunk, grams	13.98	14.46	15.23
Sr in bones of trunk, grams	.00	.75	.00
Mg in bones of trunk, grams	.25	.28	.30
P in bones of trunk, grams	6.30	6.74	6.80
Total Ca in bones, grams	27.52	33.74	32.35
Total Sr in bones, grams	.00	1.59	.00
Total Mg in bones, grams	.52	.66	.66
Total P in bones, grams	12.31	15.74	14.52
Percent of dry bones as Ca	14.84	16.60	17.23
Percent of dry bones as Sr	.00	.78	.00
Percent of dry bones as Mg	.28	.32	.35
Percent of dry bones as P	6.64	7.74	7.74
Percent of weight of body represented by Ca in bones	1.42	1.80	1.44
Percent of weight of body represented by Ca and Sr in bones	....	1.89	....
Percent of weight of body represented by Mg in bones	.026	.035	.029
Percent of weight of body represented by P in bones	.63	.84	.65

DUCKS.

LOTS XIII, XIV, AND XV.

After preliminary feeding for a few weeks with a ration of low calcium content, the three rations specially contrasted were fed for twelve weeks to six ducks, two under each ration. These ducks were cross-bred birds full grown but not yet ready to lay when feeding began.

TABLE XVI.—SHELLS OF EGGS FROM DUCKS. LOTS XIII, XIV, AND XV.

		Percent of egg as shell	Percent of calcium in dry shell	Percent of strontium in dry shell	Percent of magnesium in dry shell	Percent of phosphorus in dry shell
Lot XIII	Average of 3 eggs after 11 weeks feeding........	6.8	34.30	.00	.36	.17
Lot XIV	One egg after 11 weeks feeding..		25.78	11.04	.23	....
Lot XIV	Two eggs after 4½ months feeding.	6.4	25.24	13.04	.27	....
Lot XV	Average of 8 eggs after 8 weeks feeding........	6.9	35.52	.00	.34	.15

TABLE XVII—LAYING DUCKS.

	Lot XIII Magnesium carbonate and phosphate	Lot XIV Strontium carbonate and phosphate	Lot XV Calcium carbonate and phosphate
	Average of two birds after 12 to 13 weeks feeding	One bird after six months feeding	Average of two birds after 12 weeks feeding
Percent of weight of body represented by dry bones	9.0	8.8	10.5
Percent of dry matter in fresh bones............	68.2	62.3	70.3
Percent of dry matter in fresh bones of extremities.	66.9	66.0	77.4
Percent of dry matter in fresh bones of trunk.....	69.9	58.5	63.2
Percent of weight of body represented by ash of bones..................................	4.10	3.89	4.79
Percent of ash in dry bones...................	45.7	44.3	45.6
Percent of ash in dry bones of extremities........	48.0	43.8	46.9
Percent of ash in dry bones of trunk............	42.8	44.9	43.9
Percent of fat in dry bones of extremities........	16.6	18.4	19.5
Percent of fat in dry bones of trunk............	12.8	8.0	12.6

TABLE XVIII.—LAYING DUCKS.

	LOT XIII Magnesium carbonate and phosphate	LOT XIV Strontium carbonate and phosphate	LOT XV Calcium carbonate and phosphate
	Average of two birds after 12 to 13 weeks feeding	One bird after six months feeding	Average of two birds after 12 weeks feeding
Percent of Ca in ash of bones of extremities	38.50	35.90	39.16
Percent of Sr in ash of bones of extremities	.00	3.00	.00
Percent of Mg in ash of bones of extremities	.83	.54	.47
Percent of P in ash of bones of extremities	17.80	17.41	17.54
Percent of Ca in ash of bones of trunk	37.78	35.01	38.90
Percent of Sr in ash of bones of trunk	.00	4.23	.00
Percent of Mg in ash of bones of trunk	.85	.43	.42
Percent of P in ash of bones of trunk	17.69	17.19	17.29
Ca in bones of extremities, grams	11.80	10.38	12.75
Sr in bones of extremities, grams	.00	.87	.00
Mg in bones of extremities, grams	.25	.16	.15
P in bones of extremities, grams	5.45	5.03	5.71
Ca in bones of trunk, grams	8.41	8.83	9.54
Sr in bones of trunk, grams	.00	1.07	.00
Mg in bones of trunk, grams	.19	.11	.10
P in bones of trunk, grams	3.94	4.34	4.24
Total Ca in bones, grams	20.21	19.21	22.29
Total Sr in bones, grams	.00	1.94	.00
Total Mg in bones, grams	.44	.27	.25
Total P in bones, grams	9.39	9.37	9.95
Percent of dry bones as Ca	17.45	15.72	17.79
Percent of dry bones as Sr	.00	1.59	.00
Percent of dry bones as Mg	.38	.22	.20
Percent of dry bones as P	8.11	7.67	7.94
Percent of weight of body represented by Ca in bones	1.57	1.38	1.87
Percent of weight of body represented by Ca and Sr in bones	....	1.52	....
Percent of weight of body represented by Mg in bones	.034	.019	.021
Percent of weight of body represented by P in bones	.73	.67	.84

They were fed rice, sifted corn meal, apples (without seeds, etc.), meat meal (free from bone), blood meal, butter, and a mixture of corn meal, wheat flour, rice flour, starch, wheat gluten, sugar, and gelatine with a little common salt, and had free access to white quartz glass sand and rain water. The amount of calcium in the basal ration averaged a little less than .015% of the total dry matter. The amount of magnesium was from 2.9 to 3.1 times as much as the amount of calcium.

To the ration for Lot XIII was added a mixture of equal parts of magnesium carbonate and magnesium phosphate; to the ration for Lot XIV a mixture of equal parts of strontium carbonate and phosphate, and to that for Lot XV calcium carbonate and phosphate. These added salts represented on the average 3.8% of the total dry matter of the food for Lots XIII and XV, and for Lot XIV 3.9%.

Under these rations, the ducks in Lots XIV and XV lost in weight during the first half of the feeding period but not during the last half. The ducks in Lot XIII fed magnesium salts did not lose in weight on the average during either the first or latter half of the feeding, making instead a slight gain. One duck of Lot XIV died after six weeks feeding (the cause unknown), but the other was continued under the same ration for a second feeding period of over three months after the end of the twelve weeks feeding.

Not many eggs were laid by these ducks, but, during one period of four weeks, there was in the shells of eggs produced by Lot XIII over twenty times as much calcium as was in the food for the same period. During one period of thirty days the shells of eggs produced by Lot XV contained ten times as much calcium as was in all the food for the same period aside from the added calcium salts. For brief periods of a few days much greater differences were observed. In some instances the larger part of the calcium in the eggs came from the bones, and in other instances the larger part came directly and indirectly from the calcium salts fed.

The general mineral content of the shells of some of the eggs from these ducks is shown in table XVI, and some of the results of analyses of bones are given in tables XVII and XVIII.

CONCLUSION.

Calcium and magnesium are quite generally and abundantly distributed by nature. Many hills and mountains consist in part, or

almost wholly, of compounds of these two elements in close association but varying proportion. They exist together in most soils and in plants. In the animal body they are found in abundance. Altho in the soft tissues of the body certain functions of these elements are somewhat opposite, they work together in nature in many ways. But all the data available indicate that, for certain apparently simple associated service in the body, one does not take the place of the other.

In the usual food for the hen, as ordinarily managed under modern conditions, there is considerably more magnesium than calcium altho more calcium is required by the body. At times much more calcium is needed than is supplied by the food, and must be withdrawn from the reservoir in the bones. The skeleton is more than a foundation support of inert substance. This structure, so rich in mineral matter, is not by any means composed of dead material, but is subject to growth and depletion as are the softer tissues. Biologically, the bony framework is not so very old. In the life of the earth, as in the little history of the individual organism, it has not been the first thing to appear.

Results in experiments, started a number of years ago but not completed, are not out of accord with the idea that both calcium and phosphorus are more easily taken from the bones than from dead bone ash in the food. Even with the cow, an animal especially adapted to use in bulk the coarser foods richer in lime, there is often a negative balance of calcium during most of the milking period, altho her food may be enriched by added calcium compounds as shown by Forbes and associates.

Most grain foods and some other foods are deficient in calcium, and neither the hen nor duck can consume enough of the bulky foods to meet the calcium requirement of the laying period. The mature fowl can, however, use the calcium in inorganic food materials without much apparent disadvantage. The very young fowl cannot, with advantage, so freely supply its needs from these inorganic materials in the same form. For this, as well as for other reasons, it is important to provide a regular supply of the vegetable foods, richer in calcium, in their best condition. While it is very unlikely, under any normal conditions, that the animal will lack the small amounts of calcium required to insure proper heart action and maintain essential properties of the blood, and for other such rôles

where calcium alone will serve, the store may become too small to meet promptly certain exceptional demands without harmful stress. It is not, therefore, the part of wisdom to permit this very important reservoir of mineral elements to be depleted much, especially during growth, or except in the emergencies for which it is needed and provided.

REPORT

OF THE

Department of Bacteriology.

R. S. BREED, *Bacteriologist.*

H. J. CONN, *Associate Bacteriologist.*

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G. J. HUCKER, *Assistant Bacteriologist.*

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II. Use of nitrate-reduction test in characterizing bacteria.

III. Relation between lactic acid production and bacterial growth in the souring of milk.

REPORT OF THE DEPARTMENT OF BACTERIOLOGY.

AMMONIFICATION OF MANURE IN SOIL.*

SUMMARY.

1. The statement made in a preceding bulletin that non-spore-forming bacteria are most active in manured soil has been verified. This is contrary to the generally accepted idea that spore-forming bacteria are the important ammonifiers in soil.

2. Of these non-spore-forming organisms that are especially active in manured soil, two of the most easily recognized are *Pseudomonas fluorescens* (Flügge) Migula, and *Ps. caudatus* (Wright) Conn. They have therefore been selected for special study. Detailed descriptions of these organisms are given.

3. The culture of *Ps. fluorescens* studied has been compared with other fluorescent bacteria isolated from soil, and a review of the literature relating to fluorescent bacteria has been made. It has not proved possible to fix definite limits for this species.

4. *Ps. caudatus* (Wright) Conn is the name now assigned to the organism previously denoted by one of the writers (C) as the "orange liquefying type." It is apparently identical with the organism described by Wright in 1895 and seems to be quite common in soil and water.

5. Pure cultures of *Ps. fluorescens* and *Ps. caudatus* multiply much more rapidly in sterilized manured soil than pure cultures of *Bacillus cereus* Frankland (selected as a typical spore-former).

6. When sterilized manured soil is inoculated with a mixture of these three organisms in pure culture, the two non-spore-formers immediately gain the ascendancy, *B. cereus* becoming so reduced in numbers as to be undetectable by the ordinary methods of study.

7. In field soil to which there had been no addition of organic matter for several years, *Ps. fluorescens* and *Ps. caudatus* were rarely found while *B. cereus* was a common organism.

8. When this same soil was mixed with manure and potted, *Ps. fluorescens* and *Ps. caudatus* immediately multiplied rapidly, while but small numbers of *B. cereus* spores and no active forms of *B. cereus* could be found.

9. All three of these organisms are vigorous ammonifiers when tested in pure culture.

10. The activity of the non-spore-formers and the absence of activity of the spore-formers in unsterilized manured soil leads to the conclusion that *Ps. fluorescens* and *Ps. caudatus* are important ammonifiers of manure in soil, while there is no evidence that *B. cereus* takes part in this process.

* Reprint of Technical Bulletin No. 67, April, 1919.

FOREWORD.

A recent series of papers (Technical Bulletins 57–60 of this Station) contained the results of a study of the microscopic flora of the soil. The microörganisms of soil were classified into a few large groups, some of which were further subdivided, and in a few cases the classification was carried as far as the recognition of species. This preliminary work was considered necessary before studying the different groups with the object of recognizing more of the individual species and learning their functions.

A complete study of all soil microörganisms would be an endless task and, indeed, rather unprofitable, provided the order of studying the different types were left entirely to chance. To begin a study of this kind, therefore, those organisms should be selected that are presumably important. It is difficult to judge, *a priori*, the importance of any particular microörganisms in soil, but a hint can be obtained by observing which types predominate in natural soil under conditions of considerable importance in practice. The organisms chosen for investigation in the present work were found to multiply in freshly manured soil. In such soil, ammonification and other forms of decomposition are vigorous, and there is good reason to believe that the most rapidly multiplying organisms are of practical importance. Upon adding manure to soil, several kinds of bacteria have been found to multiply strikingly, but many of them are difficult to recognize and especially difficult to describe so that others may recognize them. It has seemed unwise to make a detailed study of any organism which could not be recognized by other workers; and the work has therefore been limited for the present to two types of organisms, both of which have been identified with previously described forms.

The two bacteria investigated belong to the group of non-spore-formers (discussed in Technical Bulletin 59 as one of the three large groups of soil microörganisms) and more specially to that division of this group described as rapid liquefiers (pp. 6–9 of the above mentioned bulletin). One of them is *Pseudomonas fluorescens* (Flügge) Migula, described on page 6 of that bulletin. The second is described on page 8 of the same bulletin as the "orange liquefying type," and has now been identified as *Bacillus caudatus* Wright. As a single polar flagellum is present, it is renamed *Pseudomonas caudatus* (Wright).

The present bulletin is divided into two sections. The first shows the predominance of these two organisms in manured soil and gives the results of an investigation of their function in soil. The second gives a detailed description of the two organisms to aid in their identification by others.

H. J. CONN.

I. WHAT SOIL ORGANISMS TAKE PART IN THE AMMONIFICATION OF MANURE?

J. W. BRIGHT.

INTRODUCTION.

The importance of the ammonification process in the soil has long been recognized, altho there has been a tendency on the part of investigators to regard it as secondary in importance to nitrification in soil fertility. Gainey,[1] however, has recently claimed that the fertility of a soil is limited by processes which precede nitrification (that is ammonification), rather than by nitrification itself.

The present work has been undertaken for the purpose of determining some of the organisms which actually cause the ammonification of manure in soil under natural conditions; to ascertain the extent to which they can carry on this ammonification; and to compare them with other organisms known to possess the power of ammonifying laboratory media.

A survey of the literature suggests that the active ammonifying organisms in the soil are generally spore-formers. This idea seems to be based principally upon the conclusion reached by Marchal[2] that the spore-forming *B. mycoides* is one of the most common of the soil organisms and the one that attacks proteins most energetically. It should be noted, however, that he worked with a miscellaneous group of organisms, and of his eight most important ammonifiers only one non-spore-former, *B. fluorescens liquefaciens*, is a typical soil organism. J. G. Lipman[3] assumed that the spore-formers were important ammonifiers, as is evidenced by the fact that he referred to the *B. subtilis* group and the Streptothrices as being the most prominent ammonifying organisms numerically important in arable soils. Stephens and Withers[4] and C. B. Lipman[5] also assumed this when they decided to use *B. subtilis* as the organism with which to do their experimental work on ammonification.

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[1] Page 402 in Gainey, P. L. The significance of nitrification as a factor in soil fertility. *Soil Sci.*, 3:399–416. 1917.

[2] Marchal, E., Sur la production de l'ammoniaque dans la sol par les microbes. *Bul. Acad. Roy. Sci., Belg.*, 3 ser., 25:727–771. 1893. English transl. *Agr. Sci.*, 8:574–601. 1894.

[3] Page 256 in Lipman, J. G. Marshall's Microbiology (2nd Edition), IX + 580 pp., Philadelphia. 1912.

[4] Stephens, F. L., and Withers, W. A., *et al.* Studies in soil bacteriology, III. *Centbl. Bakt.*, II Abt., 25:64–80. 1909.

[5] Page 106 in Lipman, C. B. Toxic and antagonistic effects of salts as related to ammonification by *Bacillus subtilis*. *Bot. Gaz.*, 48:105–125. 1909.

That this idea is still held by some soil bacteriologists is shown by the fact that in a recent piece of work done by Neller[6] (an associate of J. G. Lipman), the spore-forming organisms *B. subtilis*, *B. vulgatus*, *B. mycoides*, and *B. megatherium* are used to represent, "some of the more common species of soil organisms" causing ammonification in soil.

While it is undoubtedly true that a great many spore-forming organisms are capable of very active ammonification in manured soil, yet there is good reason to doubt their activity under natural conditions. Conn [7] has already pointed out that the spore-formers probably exist in the soil almost entirely as spores rather than as vegetative cells and that their status as active ammonifiers in soil is doubtful. He further shows [8] that the non-spore-formers not only exist in the soil in great numbers but that one group of them at least have proteolytic powers. One of this group, *Pseudomonas fluorescens*, is known to be an ammonifier. This, together with the fact that the non-spore-formers have been found to be especially abundant in freshly manured soil suggests that they may be among the important soil ammonifiers. The present work was planned to test whether this assumption is correct and, if so, to obtain as rigid proof as possible of the ammonifying agency of the non-spore-forming organisms.

TECHNIC.

The soil used thruout the series of experiments was Dunkirk silty clay loam [9] secured from a plat on the Station grounds. This soil was mixed with fresh horse manure or fresh cow manure, always in the proportion of twenty parts of soil to one part of manure.

All samples were plated according to the usual methods, using at least two dilutions. The degree of dilution depended upon the character of the samples to be plated. Four plates were made from each dilution used and the average count of the four plates taken to represent the count for that dilution. Whenever possible, the count was based upon the dilution averaging between thirty and one hundred and fifty colonies per plate. In some cases, however, it was necessary to take into account plates which varied from these limits. In a few cases where plates were lost on account of contami-

[6] Page 225 in Neller, J. R. Studies on the correlation between the production of carbon dioxide and the accumulation of ammonia by soil organisms. *Soil Sci.*, 5:225–239. 1918.

[7] Conn, H. J. Are spore-forming bacteria of any significance in soil under normal conditions? N. Y. Agr. Exp. Sta., Tech. Bul. 51. 1916.

[8] Page 17 in Conn, H. J. Non-spore-forming bacteria in soil. N. Y. Agr. Exp. Sta., Tech. Bul. 59. 1917.

[9] Described according to the system of the Bureau of Soils of the U. S. Department of Agriculture. U. S. Dep't Agr. Bureau of Soils Bul. 96, pp. 1–791. 1913. See also Survey of Ontario County, N. Y., published by this Bureau, pp. 1–55. 1912.

nation or liquefaction, the count represents an average of three instead of four plates.

The medium used in all the plating was " tap-water gelatin " made by dissolving two hundred grams of " gold-label " gelatin in one liter of tap-water, adjusting the reaction to about $P_H = 6.8$, using brom thymol blue as the indicator, and clarifying with white of egg.

Nearly all of the plate counts were checked by direct microscopic examination of the soil according to the method described by Conn.[10] An infusion of the soil to be examined was made by shaking up one gram of the soil in 9.5 c. c. of a fixative prepared by dissolving 0.15 gram of gelatin in 1000 c. c. of hot water. Of this infusion, 0.01 c. c. was measured out with a capillary pipette and smeared evenly over an area of one square centimeter on a glass slide. This smear was then dried and stained with hot rose bengal for one minute.

For all pure culture studies the manured soil was placed in small Erlenmeyer flasks, 150 grams per flask. These were then plugged with cotton and sterilized in the autoclave at 15 pounds pressure for two hours. Subsequent platings proved that in this way all organisms and spores were killed. The infusion for inoculating the soil was prepared as follows: A freshly streaked culture of the organism was suspended in sterile water and the number of organisms per cubic centimeter of this infusion determined by the above microscopic method. The infusion was then diluted to the desired strength and one cubic centimeter of it introduced into each flask. The flasks were then incubated at room temperature and studied at specified intervals. All flasks were controlled by uninoculated flasks as checks.

The method used for the determination of the ammonia produced was practically that of Potter and Snyder[11] which is an adaptation of the Folin[12] aeration method. A number of alternating Kjeldahl flasks and absorption cylinders were set up in series so that a continuous current of air could be passed thru the system. Twenty-five-gram samples of the soils to be tested were placed in the Kjeldahl flasks and 200 c. c. of $N/50\ H_2SO_4$ was put in each absorption cylinder. The flasks and cylinders were so connected that the air from the end flask was driven over into its adjoining cylinder and absorbed in the standard acid. Arranged in this way each Kjeldahl flask and adjacent absorption cylinder with the connecting tubes made one

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[10] Conn, H. J. The microscopic study of bacteria and fungi in soil. N. Y. Agr. Exp. Sta., Tech. Bul. 64. 1918.

[11] Potter, R. S., and Snyder, R. S. The determination of ammonia in soils. Ia. Agr. Exp. Sta., Research Bul. 17. 1914.

[12] Folin, O. Eine neue Methode zur Bestimmung des Ammoniaks im Harne und anderen thierischen Flüssigkeiten. *Ztschr. physiol. Chem.*, 37:161-176. 1902.

complete unit and any number of these units could be connected in the series.

When the apparatus was set up and all was in readiness for the aeration, two grams of Na_2CO_3 and 50 c. c. of ammonia-free water were introduced into each Kjeldahl flask. The flasks were then tightly stoppered, and the air turned on at such a rate that about six litres of air passed through the system per minute. The aeration was continued for about two hours and the standard acid in the absorption cylinders titrated against N/50 NaOH to determine the amount of ammonia driven off from the soil. Care was taken to have the system absolutely air-tight and all rubber connections dry so that in passing from the flasks to the cylinders none of the ammonia would be absorbed by the water. Absorption in the standard acid was aided by using Folin ammonia tubes to break up the bubbles of air when they entered the absorption cylinders.

The determination of the amount of free ammonia in soil has always been a difficult one. The accuracy of the results secured is somewhat doubtful as many protein substances present in soil are readily broken up by the reagents used in determining the ammonia present. Consequently the ammonia determinations in this series of experiments cannot be regarded as absolutely true determinations of "ammonia production." Still other factors which might tend to destroy the accuracy of the determinations are: First, that the organisms themselves might utilize the ammonia as rapidly as it is produced; and second, that it might escape into the air. The latter is improbable because the ammonia would be more likely to be absorbed by the water present in the soil. Controls of sterilized manured soil were always run at the same time as the inoculated soils, and in this way it was possible to determine whether or not the organisms in the inoculated soil affected the amount of ammonia production in any way.

RELATIVE NUMBERS OF NON-SPORE-FORMING AND SPORE-FORMING BACTERIA IN FRESHLY MANURED SOIL.

Work done by Conn [13] on the flora of freshly manured soil, previous to the present series of experiments, offers striking evidence that the non-spore-forming organisms predominate in such soil. During his work, the manured soil was plated at intervals extending over a period of six months. On the third day it was found that almost 99 per ct. of the entire flora was composed of non-spore-forming organisms.

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[13] See Table III of reference given in footnote 8.

TABLE I.— PLATE COUNTS OF MANURED SOIL IN OPEN POTS.

Counts indicate number of colonies per gram of soil

TIME SINCE ADDING MANURE TO SOIL.	Total count	ACTINOMYCETES		NON-SPORE-FORMERS		SPORE-FORMERS	
		Plate-count.	Per ct.*	Plate-count.	Per ct.*	Plate-count.	Per ct.*
2 days	60,000,000	4,000,000	7.5	56,000,000	92.5	None	0.0
3 days	80,000,000	6,000,000	7.5	74,000,000	92.5	None	0.0
4 days	125,000,000	5,000,000	4.0	116,000,000	92.7	4,000,000	3.3
6 days	235,000,000	6,000,000	2.6	220,500,000	93.5	9,500,000	3.9
9 days	45,000,000	5,000,000	11.1	38,500,000	85.5	1,500,000	3.4
13 days	43,000,000	4,000,000	9.3	36,500,000	85.0	2,500,000	5.7
16 days	35,000,000	12,000,000	34.3	23,000,000	65.7	None	0.0
21 days	50,000,000	13,000,000	26.0	35,000,000	70.0	2,000,000	4.0
24 days	55,000,000	12,500,000	22.6	42,500,000	77.4	None	0.0
29 days	85,000,000	8,500,000	10.0	76,500,000	90.0	None	0.0
38 days	45,000,000	13,000,000	29.0	32,000,000	71.0	None	0.0
58 days	95,000,000	8,500,000	8.9	83,500,000	88.0	3,000,000	3.1
94 days	18,000,000	5,500,000	30.5	11,500,000	63.8	1,000,000	5.7
123 days	20,000,000	5,000,000	25.0	15,000,000	75.0	None	0.0
Average	....	....	16.3	....	81.6	....	2.1

*Percentage of total flora.

The present work on the flora of freshly manured soil includes experiments designed to verify these earlier results.

The method of procedure in these later experiments was practically the same thruout, except for a few differences in the treatment of samples. Soil was mixed with fresh horse manure or fresh cow manure and, with the exception of the first experiment, the manured soil was then divided into two portions, one of which was placed in an open pot and one in a flask plugged with cotton. In the first experiment the manured soil was kept only in open pots. The moisture content of the pots was controlled somewhat by frequent additions of water to replace that lost by evaporation, but no allowance was made for this in the flasks. Platings were made at frequent intervals at the first of each experiment and at longer intervals as the experiment proceeded. The experiment recorded in Table I was carried on under conditions exactly analogous to those under which Conn did his previous work, and its purpose was the verification of that work. The experiments recorded in Tables II and III were also carried on under similar conditions except that soil mixed with cow manure was used, as well as that mixed with horse manure, and samples were plated from plugged flasks as well as from open pots.

A survey of the results in Tables I, II and III shows that the number of non-spore-formers in the open pots of manured soil

increased rapidly for the first few days (e. g. see Table I, fifth column). In every instance the highest percentage of this group of organisms was reached within the first week after the addition of the manure, and this maximum point was never less than 92.5 per ct., while in some cases it reached 97 per ct. or more. The results in the flasks were much more erratic and, while the percentage of the non-spore-formers often ran above 90 per ct. of the flora, the lines of increase and decrease were not so well marked

TABLE II.— PLATE COUNTS OF MANURED SOIL IN OPEN POTS AND CLOSED FLASKS: EXPERIMENT WITH COW MANURE.

Counts indicate number of colonies per gram of soil

TIME SINCE ADDING MANURE TO SOIL.	Total count.	ACTINOMYCETES.		NON-SPORE-FORMERS.		SPORE-FORMERS.	
		Plate-count.	Per ct.*	Plate-count.	Per ct.*	Plate-count.	Per ct.*
OPEN POTS.							
1 day	164,000,000	9,500,000	5.6	155,000,000	94.4	None	0.0
2 days	93,000,000	10,500,000	11.2	82,500,000	88.6	Trace	0.2
3 days	98,500,000	10,000,000	12.6	86,000,000	87.2	2,000,000	0.2
4 days	127,000,000	8,500,000	7.0	117,000,000	92.2	1,000,000	0.8
5 days	55,000,000	7,500,000	14.4	46,000,000	83.8	1,000,000	1.8
7 days	490,000,000	35,000,000	6.8	450,000,000	92.0	5,000,000	1.2
8 days	251,000,000	40,000,000	15.6	208,000,000	83.2	3,000,000	1.2
9 days	52,000,000	7,000,000	12.4	45,000,000	86.7	500,000	0.9
11 days	412,000,000	190,000,000	46.1	214,000,000	52.1	7,500,000	1.8
13 days	67,000,000	16,000,000	23.1	51,000,000	76.2	500,000	0.7
15 days	395,000,000	160,000,000	40.6	232,000,000	58.7	3,500,000	0.7
17 days	245,000,000	50,000,000	22.6	186,000,000	76.0	3,500,000	1.4
Average	....	....	18.2	....	80.9	....	0.9
CLOSED FLASKS.							
1 day	27,500,000	3,750,000	18.3	22,500,000	81.7	None	0.0
2 days	64,000,000	6,000,000	10.0	57,500,000	90.0	None	0.0
3 days	67,000,000	6,000,000	9.0	59,000,000	88.2	1,800,000	2.8
4 days	81,100,000	15,000,000	18.6	64,000,000	79.0	2,000,000	2.4
5 days	101,000,000	15,000,000	14.6	84,000,000	83.1	2,000,000	2.3
7 days	42,000,000	12,500,000	28.5	29,000,000	69.2	1,000,000	2.3
8 days	65,500,000	12,000,000	18.3	53,000,000	81.0	Trace	0.7
9 days	34,500,000	9,000,000	24.3	24,000,000	69.5	1,500,000	6.2
11 days	330,000,000	290,000,000	88.3	38,000,000	11.1	2,000,000	0.6
13 days	323,000,000	300,000,000	34.4	21,000,000	65.0	2,000,000	0.6
15 days	330,000,000	225,000,000	68.8	99,000,000	30.0	6,000,000	1.2
17 days	350,000,000	190,000,000	54.2	158,000,000	45.2	2,000,000	0.6
Average	....	....	32.3	....	66.1	....	1.6

*Percentage of total flora.

TABLE II (*concluded*).

EXPERIMENT WITH HORSE MANURE.

Counts indicate number of colonies per gram of soil

TIME SINCE ADDING MANURE TO SOIL.	Total count.	ACTINOMYCETES.		NON-SPORE-FORMERS.		SPORE-FORMERS.	
		Plate-counts.	Per ct.*	Plate-count.	Per ct.*	Plate-count.	Per ct.*
OPEN POTS.							
1 day.....	300,000,000	9,500,000	3.4	290,000,000	96.6	None	0.0
2 days.....	109,000,000	18,000,000	18.4	87,000,000	79.8	2,000,000	1.8
3 days.....	157,000,000	12,000,000	7.4	144,000,000	91.7	1,500,000	0.9
4 days.....	907,500,000	22,500,000	2.8	880,000,000	97.0	2,000,000	0.2
5 days.....	775,000,000	56,000,000	7.5	713,000,000	91.6	7,500,000	0.9
7 days.....	625,000,000	65,000,000	10.4	556,000,000	89.0	4,000,000	0.6
8 days.....	67,500,000	6,000,000	8.9	61,000,000	90.4	500,000	0.7
9 days.....	480,000,000	85,000,000	17.7	392,000,000	81.7	3,000,000	0.6
11 days.....	740,000,000	115,000,000	15.6	622,000,000	84.0	3,000,000	0.4
13 days.....	376,000,000	100,000,000	26.6	273,000,000	72.6	3,000,000	0.8
15 days.....	295,000,000	95,000,000	32.2	198,000,000	67.2	2,000,000	0.6
17 days.....	1,705,000,000	700,000,000	41.0	1,000,000,000	58.7	5,000,000	0.3
Average.....	....	....	16.1	....	83.3	....	0.6
CLOSED FLASKS.							
1 day......	63,000,000	11,000,000	17.5	52,000,000	82.5	None	0.0
2 days.....	88,000,000	7,000,000	7.8	81,000,000	92.0	Trace	0.2
3 days.....	82,000,000	8,000,000	9.9	73,000,000	89.2	750,000	0.9
4 days.....	78,000,000	12,000,000	14.6	66,000,000	84.5	750,000	0.9
5 days.....	336,000,000	100,000,000	28.6	234,000,000	70.8	2,000,000	0.6
7 days.....	810,000,000	325,000,000	40.1	481,000,000	59.5	3,500,000	0.4
8 days.....	75,500,000	24,000,000	32.4	50,000,000	66.3	1,000,000	1.3
9 days.....	58,500,000	30,000,000	51.3	27,000,000	46.2	1,500,000	2.5
11 days.....	375,000,000	250,000,000	66.6	123,000,000	32.8	2,000,000	0.6
13 days.....	169,000,000	65,000,000	2.8	162,000,000	96.0	2,000,000	1.2
15 days.....	1,380,000,000	800,000,000	57.9	575,000,000	41.7	5,000,000	0.4
17 days.....	1,045,000,000	800,000,000	76.6	244,000,000	23.3	1,000,000	0.1
Average.....	....	....	33.8	....	65.4	....	0.8

* Percentage of total flora.

as they were in the pot experiments. This was undoubtedly due to the fact that conditions of aeration and moisture content were decidedly abnormal. By summarizing the three tables it was found that the non-spore-forming organisms averaged 74.1 per ct. of the entire flora in both the pots and flasks, the Actinomycetes 25.1 per ct., and the spore-formers only 0.8 per ct.

While the data accumulated in the preceding experiments indicated very strongly that the non-spore-formers were the predomi-

nating organisms in the manured soil, yet the proof was not absolute because it was based entirely upon the appearance of the colonies upon the plates. Those colonies which possessed the characteristic spreading or filamentous appearance of the typical spore-formers were classified accordingly; but some non-spore-formers may thus have been inadvertently recorded as spore-formers, or some spore-formers as non-spore-formers. A number of isolations were made,

TABLE III.— PLATE COUNTS OF MANURED SOIL IN OPEN POTS AND CLOSED FLASKS: EXPERIMENT WITH COW MANURE.

Counts indicate number of colonies per gram of soil

TIME SINCE ADDING MANURE TO SOIL.	Total count.	ACTIONMYCETES.		NON-SPORE-FORMERS.		SPORE-FORMERS.	
		Plate-count.	Per ct.*	Plate-count.	Per ct.*	Plate-count.	Per ct.*
			OPEN POTS.				
1 day	58,000,000	8,000,000	13.8	48,000,000	82.7	2,000,000	3.5
2 days	73,250,000	5,000,000	6.7	68,000,000	93.1	Trace	0.2
3 days	497,500,000	15,000,000	3.0	482,500,000	97.0	None	0.0
4 days	93,000,000	12,000,000	12.9	81,000,000	87.1	None	0.0
5 days	102,500,000	12,500,000	12.2	90,000,000	87.8	None	0.0
7 days	36,250,000	5,000,000	13.8	31,250,000	86.2	None	0.0
8 days	43,000,000	5,000,000	11.7	38,000,000	88.3	None	0.0
9 days	17,500,000	2,500,000	14.3	15,000,000	85.7	None	0.0
10 days	40,000,000	12,000,000	30.0	28,000,000	70.0	None	0.0
12 days	96,000,000	15,000,000	15.7	81.000,000	84.3	None	0.0
15 days	187,000,000	20,000,000	10.7	167,000,000	89.3	None	0.0
18 days	75,000,000	20,000,000	26.7	55,000,000	73.3	None	0.0
21 days	64,000,000	10,000,000	14.4	52,500,000	83.3	1,500,000	2.3
Average	....	....	14.3	....	85.3	....	0.4
			CLOSED FLASKS.				
1 day	51,000,000	8,000,000	15.7	41,500,000	81.4	1,500,000	2.9
2 days	42,000,000	6,000,000	14.3	35,500,000	84.5	500,000	1.2
3 days	295,000,000	10,000,000	3.4	285,000,000	96.6	None	0.0
4 days	68,000,000	30,000,000	44.1	38,000,000	55.9	None	0.0
5 days	44,000,000	11,000,000	25.5	33,000,000	74.5	None	0.0
7 days	27,000,000	10,000,000	37.6	17,000,000	62.4	None	0.0
8 days	45,500,000	23,000,000	50.6	22,500,000	49.4	None	0.0
9 days	31,000,000	20,000,000	64.6	11,000,000	35.4	None	0.0
10 days	36,500,000	17,500,000	48.0	19,000,000	52.0	None	0.0
12 days	49,500,000	20,000,000	40.4	29,500,000	59.6	None	0.0
15 days	53,000,000	25,000,000	47.2	28,000,000	52.8	None	0.0
18 days	125,000,000	65,000,000	52.0	60,000,000	48.0	None	0.0
21 days	46,000,000	25,000,000	54.4	21,000,000	45.6	None	0.0
Average	....	....	38.3	....	61.4	....	0.3

* Percentage of total flora.

TABLE III (*concluded*).

EXPERIMENT WITH HORSE MANURE.

Counts indicate number of colonies per gram of soil.

TIME SINCE ADDING MANURE TO SOIL.	Total count.	ACTINOMYCETES.		NON-SPORE-FORMERS.		SPORE-FORMERS.	
		Plate-count.	Per ct.*	Plate-count.	Per ct.*	Plate-count.	Per ct.*
OPEN POTS.							
1 day	110,000,000	12,000,000	10.9	97,000,000	88.1	750,000	1.0
2 days	120,000,000	7,500,000	6.3	111,000,000	92.5	1,500,000	1.2
3 days	195,000,000	10,000,000	5.0	185,000,000	95.0	None	0.0
4 days	150,000,000	15,000,000	10.0	135,000,000	90.0	None	0.0
5 days	122,000,000	12,000,000	9.9	110,000,000	90.1	None	0.0
7 days	95,000,000	15,000,000	5.3	80,000,000	94.7	None	0.0
8 days	300,000,000	35,000,000	11.7	265,000,000	88.3	None	0.0
9 days	30,500,000	5,000,000	16.4	25,500,000	83.6	None	0.0
10 days	59,000,000	5,000,000	8.5	54,000,000	91.5	None	0.0
12 days	48,000,000	20,000,000	41.7	28,000,000	58.3	None	0.0
15 days	242,500,000	40,500,000	16.1	202,000,000	83.9	None	0.0
18 days	130,000,000	35,000,000	26.9	95,000,000	73.1	None	0.0
21 days	117,500,000	30,000,000	25.5	87,000,000	74.0	500,000	0.5
Average			14.9		84.9		0.2
CLOSED FLASKS.							
1 day	40,000,000	4,500,000	11.2	34,500,000	86.2	1,000,000	2.5
2 days	182,500,000	8,000,000	4.3	171,500,000	94.0	1,000,000	1.7
3 days	82,500,000	10,000,000	12.1	72,500,000	87.9	None	0.0
4 days	75,000,000	22,500,000	30.0	52,500,000	70.0	None	0.0
5 days	33,000,000	13,000,000	39.4	20,000,000	60.6	None	0.0
7 days	515,000,000	375,000,000	72.9	140,000,000	27.1	None	0.0
8 days	605,000,000	150,000,000	24.8	455,000,000	75.2	None	0.0
9 days	62,500,000	32,000,000	51.2	30,500,000	48.8	None	0.0
10 days	780,000,000	600,000,000	76.9	180,000,000	23.1	None	0.0
12 days	67,000,000	50,000,000	74.6	17,000,000	25.4	None	0.0
15 days	43,500,000	27,500,000	63.2	16,000,000	36.8	None	0.0
18 days	100,000,000	65,000,000	65.0	35,000,000	35.0	None	0.0
21 days	40,000,000	30,000,000	75.0	10,000,000	25.0	None	0.0
Average			46.2		53.5		0.3

* Percentage of total flora.

therefore, from the plates poured during the series of experiments described above. All colonies which looked like spore-formers were transferred to agar slants, as were also a representative number of colonies of other types. About 97 per ct. of these cultures grew and were subsequently examined under the microscope for spore-formation.

Table IV, which contains the recorded data from this experiment, shows that of the 254 organisms from the open pots which grew after

TABLE IV.— DATA REGARDING THE ISOLATION OF ORGANISMS FROM MANURED SOIL.

SOURCE.			OPEN POTS.					CLOSED FLASKS.				
Sample No.	Kind of manure.	Days since adding manure.	Total count.	No. of organisms isolated.	No. which grew on agar.*	No. of non-spore-formers.	No. of spore-formers.	Total count.	No. of organisms isolated.	No. which grew on agar.	No. of non-spore-formers.	No. of spore-formers.
1	Horse	6	251,000,000	34	32	30	2	317,000,000	......	......	......	......
2	Horse	10	89,000,000	20	20	20	0	95,000,000	......	......	......	......
		27	73,000,000	25	24	21	3	42,000,000	......	......	......	......
3	Horse	22	37,000,000	20	20	19	1	78,000,000	17	17	17	0
		27	30,000,000	27	27	27	0	15,000,000	5	5	5	0
4	Cow	9	231,000,000	37	36	35	1	....	......	......	......	......
5	Cow	11	....	......	......	......	......	136,000,000	41	41	39	2
6	Cow	23	174,000,000	35	33	33	0	143,500,000	35	34	34	0
7	Cow	3	98,500,000	15	14	14	0	67,000,000	14	14	14	0
8	Horse	8	67,500,000	7	6	6	0	75,500,000	9	9	9	0
9	Cow	8	251,000,000	8	8	8	0	65,500,000	4	4	4	0
10	Horse	24	1,705,000,000	13	11	11	0	1,045,000,000	15	15	15	0
11	Cow	24	245,000,000	11	10	10	0	350,000,000	9	9	8	1
12	Horse	8	300,000,000	3	3	3	0	605,000,000	5	4	4	0
13	Cow	8	43,000,000	10	10	10	0	45,500,000	8	8	8	0
			Totals	265	254	247	7		162	160	157	3

*Plain nutrient agar was used as medium for isolated colonies.

isolation, only 2.8 per ct. were spore-formers, and of the 160 organisms from the flasks which grew after isolation, only 1.8 per ct. were spore-formers; and this despite the fact that a special effort was made to include all those colonies whose appearance suggested that they might be spore-forming organisms.

GROWTH OF PSEUDOMONAS FLUORESCENS AND PS. CAUDATUS COMPARED WITH THE GROWTH OF BACILLUS CEREUS IN STERILIZED MANURED SOILS.

The organisms selected for the rest of the work were two non-spore-formers, *Pseudomonas fluorescens* (Flügge) Migula and *Pseudomonas caudatus* (Wright) Conn, and a spore-former *Bacillus cereus* Frankland.[14] The first two organisms are described in the second paper of the bulletin, and were chosen because of the frequency of their occurrence in manured soil. *B. cereus*, which is a typical spore-former occurring in soil [15] was selected for the purpose of comparison with these organisms.

SOIL INOCULATED WITH THE THREE ORGANISMS SEPARATELY.

In the series of experiments (see Tables V and VI) designed to show the relative rates of growth of the three organisms under investigation, manured soil was sterilized in flasks and inoculated with pure cultures in suspensions of carefully determined strength. Samples from each series were plated at similar intervals and an effort was made to make all results comparable. Microscopic counts were made of all the samples of soil inoculated with *B. cereus* in order to determine the number of vegetative cells actually present in the soil. As *Ps. fluorescens* and *Ps. caudatus*, on the other hand, grow well on plates, form no spores, and show no tendency to clump, a microscopic count of them was not so important as the plate count; and since they are so small as to be easily overlooked under the microscope a microscopic count proved even less accurate than the plate count. The results as set forth in Table VI, Experiment I show that *Ps. caudatus* increased from a 13,300,000 plate count on the day of inoculation to a 1,720,000,000 count seven days later, or an increase of 132 times the original inoculation. The initial plate count of *Ps. fluorescens* in Experiment I was 4,390,000, and

[14] As identified by Conn, H. J., this organism agrees with the description on page 278 of Chester's Manual of Determinative Bacteriology and the description of Ford, W. W., *et al.*, in "Studies on aerobic spore-bearing non-pathogenic bacteria," *Jour. Bact.*, 1:273–320, 493–534. 1916.

[15] Laubach, C. A., and Rice, J. L. Spore-bearing bacteria in soil. *Jour. Bact.*, 1:513–518. 1916. This paper is included in the studies of Ford, W. W., *et al.*, as given in footnote 14.

Conn, H. J. Spore-forming bacteria in soil. N. Y. Agr. Exp. Sta., Tech. Bul. 58. 1917.

TABLE V.— MULTIPLICATION OF B. CEREUS INOCULATED INTO STERILE MANURED SOIL.

Figures indicate numbers per gram of soil.

TIME SINCE INOCULATION.	Plate count.	MICROSCOPIC COUNT.			
		GROUPS.		INDIVIDUALS.	
		Vegetative cells.	Spores.	Vegetative cells.	Spores.
EXPERIMENT I.					
0 days	....	....	....	*1,800,000	None
3 days	3,000,000	5,000,000	19,000,000	9,000,000	23,000,000
5 days	1,750,000	6,000,000	6,000,000	3,000,000	6,000,000
7 days	15,000,000	36,000,000	17,000,000	50,000,000	18,000,000
EXPERIMENT II.†					
0 days	....	....	....	*1,300,000	None
5 days	2,600,000	....	....	....	....
8 days	4,000,000	....	....	....	....
17 days	7,000,000	....	....	....	....
23 days	12,000,000	....	....	....	....
EXPERIMENT III.					
0 days	....	....	....	*116,000	None
2 days	4,000,000	2,000,000	18,000,000	13,000,000	33,000,000
4 days	5,000,000	2,500,000	11,500,000	10,500,000	16,500,000
6 days	10,500,000	2,500,000	16,000,000	15,500,000	19,000,000
8 days	10,000,000	2,500,000	14,500,000	7,500,000	18,000,000
EXPERIMENT IV.					
0 days	....	....	....	*103,000	None
2 days	8,000,000	2,000,000	5,000,000	11,500,000	30,000,000
4 days	5,000,000	1,500,000	12,000,000	13,500,000	24,500,000
6 days	12,500,000	2,500,000	11,500,000	9,500,000	27,500,000
8 days	14,000,000	1,500,000	9,000,000	4,500,000	27,000,000
11 days	17,000,000	2,000,000	13,000,000	6,000,000	19,000,000
12 days	15,000,000	2,500,000	12,000,000	7,000,000	21,000,000
14 days	21,000,000	2,500,000	15,500,000	6,500,000	26,500,000
16 days	56,000,000	3,000,000	21,000,000	6,500,000	32,000,000

* Computed from the number of organisms in the infusion used for inoculation.
† No microscopic count made.

on the seventh day the count was 475,000,000, an increase of 110 times the original count. *B. cereus*, on the other hand, showed a much lower rate of increase and developed from an initial plate count of 1,800,000 (see Table V, Experiment I) to a count of 15,000,000 on the seventh day, an increase of 8.3 times the original inoculation. The microscopic count on the seventh day showed 36,000,000 vegetative cells, an increase of 20 times the initial count.

In the four series *Ps. caudatus* showed its greatest increase in Experiment III (see Table VI) on the ninth day when it showed a count 913 times greater than its initial count; *Ps. fluorescens* registered its greatest increase in Experiment IV on the fourteenth day when it showed a count 530 times higher than the original count; and *B. cereus* made its greatest increase in Experiment IV (see Table V) on the sixteenth day, showing a count 29 times higher than the original count.

VI.—MULTIPLICATION OF NON-SPORE-FORMERS INOCULATED INTO STERILE MANURED SOIL

Figures indicate numbers per gram of soil.

TIME SINCE INOCULATION.	PS. FLUORESCENS.			PS. CAUDATUS.		
	Plate count.	MICROSCOPIC COUNT.		Plate count.	MICROSCOPIC COUNT.	
		Groups.	Individuals.		Groups.	Individuals.
			EXPERIMENT I.			
0 days	...	...	*4,390,000	...	...	*13,300,000
3 days	260,000,000	197,000,000	204,000,000	665,000,000	492,000,000	608,000,000
5 days	185,000,000	133,000,000	152,000,000	4,800,000,000	1,340,000,000	1,614,000,000
7 days	475,000,000	259,000,000	325,000,000	1,720,000,000	1,105,000,000	1,254,000,000
			EXPERIMENT II.†			
0 days	...	...	*3,300,000	...	...	*6,600,000
5 days	145,000,000	...	...	2,700,000,000	...	...
8 days	160,000,000	...	...	1,500,000,000	...	...
13 days	200,000,000	...	...	No count	...	...
17 days	210,000,000	...	...	4,000,000,000	...	...
23 days	300,000,000	...	...	No count	...	...
			EXPERIMENT III.			
0 days	...	...	*1,600,000	...	...	*1,600,000
2 days	140,090,000	79,000,000	88,000,000	1,440,000,000	728,000,000	728,000,000
4 days	260,000,000	173,000,000	188,000,000	1,340,000,000	760,000,000	784,000,000
9 days	315,000,000	236,000,000	247,000,000	1,460,000,000	736,000,000	802,000,000
11 days	350,000,000	310,000,000	328,000,000	1,190,000,000	794,000,000	822,000,000
			EXPERIMENT IV.†			
0 days	...	...	*1,000,000	...	...	*1,090,000
2 days	102,000,000	...	...	114,000,000	...	...
4 days	390,000,000	...	...	181,000,000	...	...
6 days	395,000,000	...	...	194,000,000	...	...
8 days	470,000,000	...	...	220,500,000	...	...
10 days	375,000,000	...	...	205,000,000	...	...
12 days	460,000,000	...	...	220,000,000	...	...
14 days	530,000,000	...	...	269,000,000	...	...
15 days	470,000,000	...	...	171,500,000	...	...

* Computed from the number of organisms in the infusion used for inoculation.
† No microscopic count made.

SOIL INOCULATED WITH A MIXTURE OF THE THREE ORGANISMS.

Table VII shows the results of placing the three organisms in competition one with another by inoculating flasks of sterile manured soil with all of them together. Infusions were made from fresh

TABLE VII. — PLATE COUNTS OF STERILE MANURED SOIL INOCULATED WITH A MIXTURE OF ONE SPORE-FORMER AND TWO NON-SPORE-FORMERS.

Figures indicate number of colonies per gram of soil.

TIME SINCE INOCULATION.	EXPERIMENT I.			EXPERIMENT II.			EXPERIMENT III.		
	Pseudomonas caudatus.	Pseudomonas fluorescens.	Bacillus cereus.	Pseudomonas caudatus.	Pseudomonas fluorescens.	Bacillus cereus.	Pseudomonas caudatus.	Pseudomonas fluorescens.	Bacillus cereus.
0 day	*250,000	*100,000	B. cereus did not appear on the plates	*180,000	*30,000	*50,000	*1,380,000	*240,000	*680,000
1 day	None	194,000,000		10,000,000	23,000,000	B. cereus did not appear on the plates.	600,000	1,300,000	B. cereus did not appear on the plates.
2 days	None	188,000,000		89,000,000	106,000,000		No test	No test	
3 days	None	260,000,000		297,000,000	560,000,000		24,000,000	6,900,000	
4 days	80,000,000	240,000,000		490,000,000	150,000,000		129,000,000	15,000,000	
5 days	480,000,000	150,000,000		575,000,000	150,000,000		No test	No test	
6 days	390,000,000	220,000,000		460,000,000	180,000,000		No test	No test	
7 days	570,000,000	135,000,000		1,190,000,000	250,000,000		No test	No test	
8 days	710,000,000	100,000,000		1,090,000,000	200,000,000		No test	No test	
9 days	770,000,000	110,000,000		1,020,000,000	130,000,000		No test	No test	
10 days	865,000,000	72,500,000		920,000,000	90,000,000		No test	No test	
11 days	920,000,000	75,000,000		900,000,000	80,000,000		580,000,000	60,000,000	
12 days	710,000,000	50,000,000		680,000,000	90,000,000		610,000,000	80,000,000	
13 days	880,000,000	80,000,000		950,000,000	120,000,000		650,000,000	65,000,000	
14 days	810,000,000	110,000,000		750,000,000	85,000,000		725,000,000	60,000,000	
15 days	870,000,000	90,000,000		860,000,000	80,000,000		No test	No test	
16 days	820,000,000	80,000,000		750,000,000	80,000,000		No test	No test	
17 days	865,000,000	80,000,000		180,000,000	30,000,000		No test	No test	
18 days	960,000,000	70,000,000		600,000,000	90,000,000		320,000,000	50,000,000	
19 days	730,000,000	80,000,000		580,000,000	110,000,000		250,000,000	20,000,000	
20 days	800,000,000	65,000,000		605,000,000	90,000,000		170,000,000	None	
21 days	....	....		360,000,000	150,000,000		270,000,000	50,000,000	
22 days	....	....		425,000,000	150,000,000		310,000,000	65,000,000	
23 days	....	....		590,000,000	140,000,000		No test	No test	
24 days	....	....		735,000,000	100,000,000		300,000,000	70,000,000	
25 days	....	....		730,000,000	100,000,000		No test	No test	
26 days	....	....		No test	No test		330,000,000	75,000,000	
27 days	....	....		420,000,000	195,000,000		No test	No test	
28 days	....	....		550,000,000	80,000,000		No test	No test	
29 days	....	....		430,000,000	110,000,000		460,000,000	65,000,000	
30 days	....	....		330,000,000	50,000,000		No test	No test	
31 days	....	....		290,000,000	60,000,000		380,000,000	70,000,000	
36 days	....	....		....	....		310,000,000	60,000,000	
38 days	....	....		....	....		300,000,000	55,000,000	

* Computed from the number of organisms in the infusion used for inoculation.

cultures of each organism and the strength of these infusions determined by the microscopic method. After infusions of the proper strength had been secured, equal amounts of each were thoroly mixed and one cubic centimeter of the mixture added to the flasks containing 150 grams of sterile manured soil. These flasks were then incubated at room temperature and plates and smears made from them at regular intervals. In Experiment I, Table VII, the initial ratio between the numbers of organisms of *Ps. fluorescens*, *Ps. caudatus* and *B. cereus*, was 1:1:1; in Experiment II the initial ratio was 1:8:33; in Experiment III the initial ratio was 1:7:33. Altho *B. cereus* was as abundant as the other organisms in Experiment I and was much more numerous than they in the later experiments, it failed to appear upon any of the plates poured. The non-spore-forming organisms multiplied very rapidly, and in Experiment II, *Ps. fluorescens* developed from an initial count of 30,000 to a maximum count of 560,000,000 on the third day, an increase of over 18,500 times its count at the time of inoculation. In the same experiment, *Ps. caudatus* developed from an initial count of 180,000 to a maximum count of 1,190,000,0000 on the seventh day, an increase of 6,600 times its count at the time of inoculation. The microscopic examination of the smears made during this series of experiments showed that the vegetative cells of *B. cereus* rapidly decreased in numbers and in a few days the organism could be identified only in the spore form while the non-spore-formers, especially *Ps. caudatus*, showed a steady increase in numbers for several days.

The results as recorded in this series of experiments indicate quite clearly that the non-spore-forming organisms, *Ps. fluorescens* and *Ps. caudatus*, rapidly gain the ascendency over *B. cereus* when placed in competition with it in sterile freshly manured soil. The vegetative cells of *B. cereus* apparently soon sporulate and remain in the resting condition.

RELATIVE NUMBERS OF THE ORGANISMS IN QUESTION IN A SOIL BEFORE AND AFTER ADDING MANURE.

Tables VIII, IX, X and XI record data which show the relative numbers of *Ps. fluorescens*, *Ps. caudatus*, and *B. cereus* in soil in which no ammonification is occurring, and in the same soil after manure has been added and decomposition is occurring rapidly. Table VIII [16] contains the data obtained as the result of analyses of an untreated field soil from Plat I made at intervals during a period of three years. It will be noted that during that time, the non-spore-forming *Ps. caudatus* appeared only once and then in comparatively small numbers; *Ps. fluorescens* appeared nine times and

[16] The data given in this table were obtained by Conn, H. J., in his earlier work. Much of it has already been used in his soil flora studies. N. Y. Agr. Exp. Sta., Tech. Bul. 57-60. 1917.

only twice constituted more than 1 per ct. and never more than 2.5 per ct. of the total flora. It is also a significant fact that the spore-forming *B. cereus* was always present and made up from 0.55 per ct. to 4.4 per ct. of the total flora. Another plating made just previous to the present work showed that the organisms were present as follows: *Ps. fluorescens* 2 per ct., *Ps. caudatus* less than 0.1 per ct. and *B. cereus* 2.4 per ct.

Tables IX, X and XI record the results of platings made from samples of the same soil after being treated with fresh manure. Examination of these tables shows that either *Ps. fluorescens* or *Ps. caudatus* or both almost invariably appeared on every sample plated and often constituted as high as 15 or 20 per ct. of the entire flora while *B. cereus* was very seldom observed and, in fact, very few spore-formers of any type were recognized. It must be borne in mind that these data were obtained from soil in which it was definitely determined that decomposition processes were occurring.

TABLE VIII.— COMPARISON BETWEEN NUMBERS OF B. CEREUS AND THE NUMBERS OF CERTAIN NON-SPORE-FORMERS IN PLAT I.— SOIL UNTREATED.

Figures indicate number of colonies per gram of soil.

DATE.	Total count.	Bacillus cereus.	Pseudomonas fluorescens.	Pseudomonas caudatus.
1912.				
Sept. 23	38,250,000	350,000	*None	60,000
Oct. 25	17,000,000	150,000	150,000	None
Dec. 3	35,000,000	200,000	None	None
Dec. 17	23,500,000	No count	No count	No count
1913.				
Jan. 15	17,500,000	200,000	100,000	None
Feb. 5	27,500,000	350,000	None	None
Feb. 14	54,000,000	300,000	300,000	None
Mar. 11	29,200,000	400,000	None	None
April 4	27,000,000	400,000	200,000	None
July 10	22,600,000	500,000	None	None
Nov. 26	15,000,000	350,000	None	None
Dec. 15	12,400,000	200,000	Trace	None
1914.				
Jan. 16	16,150,000	700,000	None	None
Jan. 30	29,300,000	500,000	Trace	None
Feb. 7	26,700,000	400,000	None	None
Feb. 26	38,500,000	600,000	Trace	None
April 15	19,400,000	350,000	None	None
April 29	16,100,000	450,000	None	None
Aug. 7	**	200,000	None	None
Aug. 19	23,400,000	175,000	600,000	None
1917.				
May 4	12,500,000	300,000	250,000	None

* Dilution so great that no colonies appeared on the plates.
** Count lost on account of liquefaction.

TABLE IX.— COMPARISON BETWEEN NUMBERS OF BACILLUS CEREUS AND NUMBERS OF CERTAIN NON-SPORE-FORMERS IN SOIL FROM PLAT I.— SOIL MANURED AND KEPT IN THE LABORATORY.

Counts indicate number of colonies per gram of soil.

Time Since Adding Manure to Soil.	Pot.						Flask.					
	Total count.	Ps. fluorescens.		Ps. caudatus.		B. cereus.	Total count.	Ps. fluorescens.		Ps. caudatus.		B. cereus.
		Plate count.	Per ct.*	Plate count.	Per ct.*			Plate count.	Per ct.*	Plate count.	Per ct.*	
EXPERIMENT I.						B. cereus did not appear on the plates.						B. cereus did not appear on the plates.
7 days	251,000,000	10,000,000	4.0	None	0.0		317,000,000	None	0.0	9,000,000	2.8	
16 days	105,000,000	6,000,000	5.7	47,000,000	44.8		99,000,000	10,000,000	9.9	50,000,000	50.5	
EXPERIMENT II.												
3 days	89,000,000	5,000,000	5.7	25,000,000	28.2		142,000,000	10,000,000	7.0	35,000,000	25.0	
13 days	177,000,000	7,000,000	4.0	50,000,000	28.3		485,000,000	75,000,000	15.4	35,000,000	7.2	
20 days	73,000,000	3,000,000	4.2	10,000,000	13.7		43,000,000	2,000,000	4.7	4,000,000	9.5	
EXPERIMENT III.												
5 days	233,000,000	17,000,000	7.3	50,000,000	21.4		145,000,000	10,000,000	6.9	20,000,000	13.8	
15 days	37,000,000	2,000,000	5.4	5,000,000	13.5		78,000,000	4,000,000	5.1	3,000,000	3.9	
20 days	30,000,000	2,000,000	6.6	1,000,000	3.4		22,000,000	1,000,000	4.5	5,000,000	2.2	
EXPERIMENT IV.												
4 days	231,000,000	4,000,000	1.7	18,000,000	7.8		136,000,000	17,000,000	12.5	35,000,000	25.7	
23 days	174,000,000	No count	.....	No count	.....		143,500,000	No count	.....	No count	.....	
Average of all experiments	....	....	4.9	....	17.9	....	....	....	7.3	....	15.6	....

* Per ct. of total flora.

TABLE X.— COMPARISON BETWEEN NUMBERS OF B. CEREUS AND NUMBERS OF CERTAIN NON-SPORE-FORMERS IN SOIL FROM PLAT I.— SOIL MANURED AND KEPT IN THE LABORATORY.

Counts indicate number of colonies per gram of soil.

TIME SINCE ADDING MANURE TO SOIL.	POT.					FLASK.				
	Total count.	PS. FLUORESCENS.		PS. CAUDATUS.		Total count.	PS. FLUORESCENS.		PS. CAUDATUS.	
		Plate-count.	Per ct.*	Plate-count.	Per ct.*		Plate-count.	Per ct.*	Plate-count.	Per ct.*
				HORSE MANURE.						
1 day	300,000,000	Trace	0.1	Trace	0.1	63,000,000	None	0.0	None	0.0
2 days	109,000,000	2,000,000	1.8	None	0.0	88,000,000	1,000,000	1.2	None	0.0
3 days	157,000,000	7,500,000	4.7	None	0.0	82,000,000	1,500,000	1.8	None	0.0
4 days	907,500,000	30,000,000	3.3	None	0.0	78,000,000	2,000,000	2.6	None	0.0
5 days	775,000,000	25,000,000	3.2	Trace	0.1	336,000,000	5,000,000	1.5	None	0.0
7 days	625,000,000	20,000,000	3.2	20,000,000	3.2	810,000,000	2,500,000	0.3	None	0.0
8 days	67,500,000	750,000	1.1	1,250,000	1.8	75,500,000	None	0.0	500,000	0.6
9 days	480,000,000	12,000,000	2.5	15,000,000	3.1	58,500,000	None	0.0	None	0.0
11 days	740,000,000	62,000,000	8.4	8,000,000	1.1	375,000,000	750,000	0.2	750,000	0.2
13 days	376,000,000	22,500,000	6.0	7,500,000	2.0	169,000,000	1,000,000	0.6	1,000,000	0.6
15 days	295,000,000	5,000,000	1.7	4,000,000	1.3	1,380,000,000	2,500,000	0.1	5,000,000	0.4
17 days	1,705,000,000	20,000,000	1.1	50,000,000	2.9	1,045,000,000	1,000,000	0.9	None	0.0
Average	....	....	3.1	....	1.3	....	....	0.8	....	0.15
				COW MANURE.						
1 day	164,000,000	Trace	0.1	Trace	0.1	27,500,000	Trace	0.1	None	0.0
2 days	93,000,000	Trace	0.2	Trace	0.2	64,000,000	2,000,000	3.1	Trace	0.1
3 days	98,500,000	500,000	0.5	None	0.0	67,000,000	1,800,000	2.7	1,000,000	1.5
4 days	127,000,000	3,500,000	2.7	None	0.0	81,000,000	None	0.0	None	0.0
5 days	55,000,000	500,000	0.9	None	0.0	101,000,000	None	0.0	None	0.0
7 days	490,000,000	1,000,000	0.2	None	0.0	42,000,000	1,500,000	3.6	None	0.0
8 days	251,000,000	1,500,000	0.6	None	0.0	65,500,000	Trace	0.2	None	0.0
9 days	52,000,000	1,000,000	1.9	500,000	0.9	34,500,000	None	0.0	None	0.0
11 days	412,000,000	2,500,000	0.6	7,500,000	1.9	330,000,000	Trace	0.1	None	0.0
13 days	67,000,000	Trace	0.2	None	0.0	323,000,000	Trace	0.1	None	0.0
15 days	395,000,000	5,000,000	1.3	10,000,000	2.6	330,000,000	Trace	0.1	1,000,000	0.3
17 days	245,000,000	None	0.0	1,000,000	0.2	350,000,000	2,000,000	0.7	None	0.0
Average	....	....	0.8	....	0.5	....	....	0.9	....	0.2

* Per ct. of total flora.

TABLE XI.— COMPARISON BETWEEN NUMBERS OF B. CEREUS AND NUMBERS OF CERTAIN NON-SPORE-FORMERS IN SOIL FROM PLAT I.— SOIL MANURED AND KEPT IN THE LABORATORY.

Counts indicate number of colonies per gram of soil.

Time Since Adding Manure to Soil.	Pot.					Flask.				
	Total count.	Ps. fluorescens.		Ps. caudatus.		Total count.	Ps. fluorescens.		Ps. caudatus.	
		Plate-count.	Per ct.*	Plate-count.	Per ct.*		Plate-count.	Per ct.*	Plate-count.	Per ct.*
				Horse Manure.						
1 day	110,000,000	2,000,000	1.8	1,500,000	1.4	40,000,000	750,000	1.8	1,000,000	2.5
2 days	120,000,000	6,000,000	5.0	5,000,000	4.2	182,500,000	10,000,000	5.5	25,000,000	13.7
3 days	195,000,000	4,000,000	2.1	35,000,000	18.0	82,500,000	2,000,000	2.4	15,000,000	18.2
4 days	150,000,000	6,000,000	4.0	13,000,000	8.7	75,000,000	3,000,000	4.0	5,000,000	6.7
5 days	122,000,000	4,000,000	3.3	8,000,000	6.6	33,000,000	2,000,000	6.1	4,000,000	12.2
7 days	95,000,000	10,000,000	10.5	5,000,000	5.2	515,000,000	5,000,000	1.0	15,000,000	3.0
8 days	300,000,000	55,000,000	18.3	40,000,000	13.3	605,000,000	35,000,000	5.8	15,000,000	2.5
9 days	30,500,000	1,500,000	4.9	1,500,000	4.9	62,500,000	None	0.0	1,000,000	1.6
10 days	59,000,000	1,500,000	2.5	1,500,000	2.5	780,000,000	Trace	0.1	1,000,000	0.1
12 days	48,000,000	3,000,000	6.3	5,000,000	10.4	67,000,000	1,000,000	1.5	None	0.0
15 days	242,500,000	25,000,000	10.3	22,500,000	9.3	43,500,000	None	0.0	1,000,000	2.3
18 days	130,000,000	7,500,000	5.8	7,500,000	5.8	100,000,000	2,000,000	2.0	None	0.0
21 days	117,500,000	2,000,000	1.9	2,000,000	1.9	40,000,000	None	0.0	None	0.0
Average	....	....	5.9	....	7.1	....	....	2.3	....	4.8
				Cow Manure.						
1 day	58,000,000	None	0.0	750,000	1.3	51,000,000	800,000	1.6	2,000,000	3.9
2 days	73,250,000	7,500,000	10.3	5,000,000	6.8	42,000,000	1,000,000	2.4	2,000,000	4.8
3 days	497,500,000	10,000,000	2.0	30,000,000	6.1	295,000,000	25,000,000	8.5	12,000,000	4.2
4 days	93,000,000	2,000,000	2.1	7,500,000	8.1	68,000,000	2,000,000	2.9	3,000,000	4.4
5 days	102,500,000	3,500,000	3.4	1,000,000	1.0	44,250,000	2,000,000	4.5	3,500,000	7.9
7 days	36,250,000	1,000,000	2.8	None	0.0	27,250,000	None	0.0	1,000,000	4.0
8 days	43,000,000	2,000,000	4.6	None	0.0	45,500,000	None	0.0	1,000,000	2.2
9 days	17,500,000	None	0.0	None	0.0	31,000,000	None	0.0	2,000,000	6.4
10 days	40,000,000	None	0.0	1,000,000	2.5	36,500,000	500,000	1.4	5,000,000	14.0
12 days	96,000,000	3,500,000	3.7	4,500,000	4.7	49,500,000	1,000,000	2.0	5,000,000	10.0
15 days	187,000,000	7,500,000	4.0	4,000,000	2.1	53,000,000	1,500,000	2.8	1,000,000	1.9
18 days	75,000,000	10,000,000	13.3	2,000,000	2.7	125,000,000	10,000,000	8.0	2,000,000	1.6
21 days	64,000,000	1,500,000	2.3	5,000,000	7.8	46,000,000	Trace	0.2	None	0.0
Average	....	....	3.7	....	3.3	....	....	2.6	....	5.0

* Per ct. of total flora.

AMMONIFICATION BY THE ORGANISMS IN QUESTION IN STERILIZED MANURED SOIL.

INOCULATED WITH THE THREE ORGANISMS SEPARATELY.

Table XII contains the data secured when the soil inoculated with pure cultures of each of the three organisms separately were subjected to the ammonia test previously described (p. 7). All of the organisms were found to be ammonifiers and so far as the individual organisms are concerned the data indicate that, per organism, *B. cereus* is the most powerful ammonifier of the three. When the plate count of *B. cereus* showed 17,000,000 colonies per gram of soil on the tenth day after inoculation, the ammonia production was 22 mgs. per 100 gms. of soil (Table XII, Experiment II). On the other hand, a plate count of a flask inoculated with *Ps. fluorescens* showed 375,000,000 colonies per gram of soil on the tenth day and an ammonia production of 20.28 mgs. per 100 gms. of soil (Table XII, Experiment II), and a plate count from a flask inoculated with *Ps. caudatus* showed 220,000,000 colonies per gram of soil on the eighth day and an ammonia production of 17.84 mgs. per 100 gms. of soil (Table XII, Experiment II). This fact does not prove, however, that *B. cereus* is an important ammonifier in unsterilized manured soil. The data already discussed (p. 18) indicating that under natural conditions the organisms of the *B. cereus* group are present in manured soil in very small numbers and that the vegetative cells quickly disappear.

INOCULATED WITH A MIXTURE OF THE THREE ORGANISMS.

Table XIII contains the data secured as the result of inoculating sterilized manured soil with a mixture of the three organisms. In Experiment I all of the organisms were inoculated in approximately equal numbers. It is a noteworthy fact that while *Ps. fluorescens* and *Ps. caudatus* showed rapid development and were always present in large numbers, *B. cereus* never developed upon the plates and showed a very rapid decrease in the number of vegetative cells present in the smears examined under the microscope.

Despite the very evident fact that *B. cereus* was not active in the samples tested, the amount of ammonia produced was quite marked. This was a strong indication that the ammonia produced was due to the activity of the only other organisms present, *Ps. fluorescens* and *Ps. caudatus*. In Experiment II the organisms were inoculated in the proportion of 1 individual of *Ps. fluorescens* to 8 of *Ps. caudatus* to 33 of *B. cereus*. Even with such a favorable start as this *B. cereus* again failed to develop on the plates and showed a rapid decrease in the number of vegetative cells present. The degree of ammonification was marked in this series also.

TABLE XII.— AMMONIA PRODUCED BY BACILLUS CEREUS AND THE NON-SPORE-FORMERS IN STERILE MANURED SOIL.

TIME SINCE INOCULATION.	B. CEREUS.			PS. CAUDATUS.			PS. FLUORESCENS.		
	Total count.	MGS. AMMONIA PER 100 GMS. OF SOIL.		Total count.	MGS. AMMONIA PER 100 GMS. OF SOIL.		Total count.	MGS. AMMONIA PER 100 GMS. OF SOIL.	
		Inoculated flask.	Blank control.		Inoculated flask.	Blank control.		Inoculated flask.	Blank control.
				EXPERIMENT I.					
2 days	4,000,000	11.24	4.6	1,400,000,000	5.12	3.4	140,000,000	5.8	1.36
4 days	5,000,000	10.92	.68	1,340,000,000	11.56	1.36	260,000,000	16.32	7.76
9 days	10,000,000	12.56	1.36	1,460,000,000	20.4	7.76	315,000,000	11.2	2.72
11 days	10,500,000	17.68	5.44	1,190,000,000	14.96	2.72	350,000,000	19.04	5.44
				EXPERIMENT II.					
2 days	8,000,000	7.12	3.04	114,000,000	7.48	4.4	102,000,000	12.76	4.24
4 days	5,000,000	12.56	5.08	181,000,000	11.88	4.4	390,000,000	15.32	4.16
6 days	12,500,000	10.2	3.4	194,000,000	10.36	5.44	395,000,000	20.00	6.44
8 days	14,000,000	15.64	2.72	220,000,000	17.84	6.12	470,000,000	20.24	6.64
10 days	17,000,000	22.00	6.24	206,000,000	17.48	5.24	375,000,000	20.28	Lost
12 days	15,000,000	18.72	3.72	220,000,000	16.12	4.4	460,000,000	17.80	6.44
14 days	21,000,000	21.68	5.08	269,000,000	16.64	4.4	530,000,000	17.32	5.44
16 days	56,000,000	17.32	4.24	171,500,000	16.48	5.24	470,000,000	14.28	6.60

TABLE XIII.— AMMONIA PRODUCED BY A MIXTURE OF BACILLUS CEREUS AND THE TWO NON-SPORE-FORMERS IN STERILE MANURED SOIL.

TIME SINCE INOCULATION.	TOTAL COUNT PER GM. OF SOIL.			MGS. AMMONIA PER 100 GMS. OF SOIL.	
	Ps. fluorescens.	Ps. caudatus.	B. cereus.	Inoculated flask.	Blank control.
		EXPERIMENT I.			
0 day..........	*2,573,000	*2,406,000	*2,380,000	....	....
4 days..........	240,000,000	80,000,000	B. cereus did not appear on the plates	19.52	5.88
6 days..........	220,000,000	390,000,000		19.56	4.8
8 days..........	100,000,000	710,000,000		20.4	5.08
11 days..........	75,000,000	920,000,000		14.96	4.76
13 days..........	80,000,000	880,000,000		21.12	5.08
15 days..........	90,000,000	870,000,000		17.52	No test
17 days..........	80,000,000	865,000,000		20.36	No test
19 days..........	80,000,000	730,000,000		19.56	No test
		EXPERIMENT II.			
0 day..........	*80,000	*640,000	*2,600,000	....	....
7 days..........	250,000,000	1,190,000,000	B. cereus did not appear on the plates	15.96	7.12
9 days..........	130,000,000	1,020,000,000		18.2	7.48
15 days..........	80,000,000	860,000,000		21.16	5.92
23 days..........	140,000,000	590,000,000		21.12	6.12
27 days..........	195,000,000	420,000,000		19.52	6.12
30 days..........	50,000,000	330,000,000		17.36	6.32
31 days..........	60,000,000	290,000,000		15.96	6.12

* Computed from the number of organisms in the infusion used for inoculation.

Thruout the entire series of ammonification experiments, a correlation seemed to exist between the time that had elapsed since the inoculation of the soil and the amount of ammonia produced rather than between the number of organisms present and the ammonia production. The tests were continued until the apex of ammonia production was apparently reached. After this point had been reached, a steady decrease in ammonia content was noted regardless of the number of organisms present. This was undoubtedly due to the depletion of available organic matter.

DISCUSSION.

The heterogeneous nature of soil, the great variety of organisms present, and the varying moisture content are all factors which make it practically impossible to carry on a study of ammonification in the soil under absolutely natural conditions. In order to control these things and secure comparable data it is necessary to bring the soil into the laboratory and study it there. This introduces a

difficulty, in that conditions governing the activities of organisms in the laboratory are generally at wide variance from conditions in the natural environment of these organisms. The reason for this variance is twofold:— First, laboratory media may often be decidedly different from the soil in which the organisms are native; and second, the organisms in pure culture, as they are generally handled in the laboratory for purposes of control, do not behave as they would in competition or in association with the other organisms normally present in the soil. These artificial conditions must be kept in mind in considering the results secured in the present work; but despite them, there was one striking relation which invariably held true, namely: the spore-forming *B. cereus* never multiplied in manured soil with any degree of rapidity while *Ps. fluorescens* and *Ps. caudatus* always did.

The data indicate that in soil where little organic matter is present and the processes of soil decomposition are practically at a standstill the spore-former *B. cereus* occurs much more often than do the non-spore-formers *Ps. fluorescens* and *Ps. caudatus*. When organic matter in the form of manure has been added to that same soil, however, and the processes of decomposition become active, the character of the flora changes entirely and *Ps. fluorescens* and *Ps. caudatus* predominate over *B. cereus*. But the proof that these non-spore-formers are the important ammonifiers in manured soil is decidedly difficult to secure.

As Conn [17] has previously pointed out, there are four points which must be established before we can show conclusively that any particular chemical transformation in the soil is due to certain organisms: "(1) The organism must be shown to be present in active form when the chemical transformation under investigation is taking place; (2) It must be shown to occur in larger numbers under such conditions than in the same soil in which the chemical change is not occurring; (3) It must be isolated from the soil and studied in pure culture; (4) The same chemical change must be produced by the organism in experimentally inoculated soil, making the test, if possible, in unsterilized soil. The fourth requirement, however, can ordinarily be carried out only by inoculating sterilized soil, a procedure which does not give rigid proof, but which is fairly conclusive if carried out in connection with the other three requirements."

The data presented above offer fairly conclusive proof that these conditions have all been fulfilled by the organisms in question. The first step in the proof may be found in Tables IX, X and XI, where it is shown that one or both of the non-spore-forming organisms in question are always present in active form in manured soil in which

[17] Conn, H. J. The proof of microbial agency in the chemical transformations of soil. *Sci.*, N. S., 46:253–255. 1917.

ammonification is occurring. That the second requirement is fulfilled is shown by Tables VIII, IX, X and XI in which it may be seen that the non-spore-formers *Ps. fluorescens* and *Ps. caudatus* occur in much greater numbers in decomposing manured soil than in the same soil before the manure has been added, and that the spore-former *B. cereus* occurs in great abundance in the soil before adding manure but disappears almost entirely after manuring. The isolation of pure cultures, the third step in the proof, needs no comment; while the fourth is fulfilled, as seen by reference to Table XII, where it is shown that pure cultures of the organisms in question have the power of ammonifying manure in soil.

On the basis of the data obtained, there are, therefore, no good reasons for believing that the spore-forming organisms play an important role in ammonifying manure in soil, and there is very good evidence that the non-spore-formers, *Ps. fluorescens* (Flügge) Migula and *Ps. caudatus* (Wright) Conn, are of primary importance in ammonification in manured soils.

II. TAXONOMIC STUDY OF TWO IMPORTANT SOIL AMMONIFIERS.

H. J. CONN.

DISTRIBUTION.

The preceding section of this bulletin is concerned with the ammonifying powers of two soil bacteria, *Pseudomonas fluorescens* (Flügge) Migula and *Ps. caudatus* (Wright) Conn. Both of them are believed to be very widely distributed in nature. There is no question as to the wide distribution of *Ps. fluorescens* because it has been described again and again by previous investigators as occurring in various localities. This, or some other similar organism, has been found most frequently in soil and in water; but has also been reported in air, butter, maple sap, and other substances. It has been observed by the present writer in practically all soils investigated, especially in soil that has been aerated or manured. *Ps. caudatus* is probably equally widely distributed; but the difficulty in recognizing it from published descriptions renders the literature concerning it of doubtful value. No references to similar organisms in soil have been found, but various writers have described yellow or orange liquefying bacteria found in water, some of which are undoubtedly the same as the organism studied here. Water was the source of Wright's *Bacillus caudatus*. The writer has observed it in water, and in many soils, especially in freshly manured soil.

To aid in the identification by others of these two organisms studied by Bright, a detailed investigation of their physiology and cultural characteristics has been made, and the characteristics observed have been compared with those described by other writers. The following paper contains a description of these characteristics and a discussion of the probable relationships of these organisms to others.

PSEUDOMONAS FLUORESCENS (FLÜGGE) MIGULA 1900, p. 886.

This organism was first described by Flügge (1886, p. 289)[1] as *Bacillus fluorescens liquefaciens*. The description is rather meager, but the organism is plainly specified as a motile short rod, liquefying gelatin rapidly with the formation of a greenish-yellow fluorescence, producing a brownish growth on potato, and occurring in water and in decomposing material. This description indicates beyond question the group of fluorescent pseudomonads, even if the exact species or

[1] Reference to bibliography is made by author's name and year following name in parenthesis.

variety is uncertain. In the later edition of this book (Kruse, 1896, Vol. II, p. 292) the organism is described more definitely with the following additional information: Size 0.3–0.5 by 1–2 micron; no spores; Gram stain negative; optimum temperature 20–25°. Kruse further makes the statement that " wenn man alle kleinen Abweichungen als Konstante Merkmale auffassen wollte, müsste man Dutzende von Arten aufstellen."

The organism is much more fully described by Lehmann and Neumann (1896, Vol. II, p. 272) under the name of *Bacterium fluorescens* (the adjective " liquefaciens " dropped to avoid a trinomial, and placed in the genus Bacterium because these authors placed only spore-formers in Bacillus). They state that it is identical with *B. pyocyaneum* in " allen wesentlichen Eigenschaften." It is described as having polar flagella. *B. pyocyaneum* is described as producing no gas from dextrose, indol, or H_2S, but as converting nitrate into nitrogen; from which it is to be assumed that *B. fluorescens* agrees in these characteristics, altho nothing definite is said on the subject except in regard to indol and H_2S. Migula (1900) placed this organism in his genus Pseudomonas created to contain the rods with polar flagella. Migula describes *Ps. fluorescens* at some length, but lays greatest stress on cultural characteristics and adds little of importance to Kruse's description. Migula gives its diameter as ca. 0.68 micron.

Many other writers have described the same or some similar organism. Many different names have been given to fluorescent bacteria from time to time, Tanner (1918) having recently stated that 95 different names had been found in a search thru the literature. Many of these names are trinomials or worse, such as *Bacillus fluorescens putidus* Flügge (1886) *B. fluorescens liquefaciens minutissimus* Unna, Tommasoli,[2] etc., but others have conformed with approved rules of nomenclature. The greater number of the fluorescent organisms have been found in water, soil, or decaying organic matter, but one of the best known forms, the *pyocyaneus* type (more correctly named *Ps. aeruginosa* (Schroeter) Migula) causes blue pus. As mentioned by Kruse (1896), there is great variation among these organisms and if each variation be taken as a constant characteristic, an almost endless variety of species could be named. This fact naturally raises the question how many of the names found in the literature are valid and how many are really synonyms, having been applied to mere physiological variations of a previously described species. Even the blue pus organism is thought by some writers to be identical with the saprophytic forms. We have not yet sufficient data to straighten out completely the resulting confusion, but a careful search thru the literature throws a little light on the matter. The information accumulated

[2] According to Eisenberg, J. (1891), p. 76.

during the present work has made it possible to review this literature more intelligently than could have been done otherwise; and it seems well, therefore, to summarize the writings of others in regard to some of the more important fluorescent organisms.

Bacterium termo (Müller) Ehrb.— This name was given by Ehrenberg (1830) to what he considered the *Monas termo* of O. F. Müller (1786). The same name was used by various writers during the next three or four decades to designate almost any motile rod found abundantly in decaying organic matter. Finally Cohn (1872, p. 196) described *B. termo* as a green fluorescent organism obtained from decomposing seeds by making a series of transfers into tubes of Cohn's solution.[3] By means of this same technic, a culture has been obtained in the course of the present work agreeing fairly well with Cohn's organism, a vigorous denitrifier,[4] differing from all other fluorescent pseudomonads investigated here. Erwin Smith (1905, p. 170), however, used this technic and obtained a green fluorescent organism differing distinctly (in failure to liquefy gelatin and in having but one instead of two flagella) from the one found in this laboratory. It seems doubtful, therefore, whether Cohn's organism actually represents one or several species; and as there is some question as to whether Cohn was justified in his emendation of the species, the name is not used in the present bulletin.

Van Iterson (1902) described a non-liquefying, fluorescent denitrifier (*B. denitrofluorescens*) which may perhaps be the same as Smith's "*B. termo*" or closely related to it. Other fluorescent, denitrifying bacteria have been described by Severin (1897 and 1909) and by Jensen (1898). It is evident, therefore, that in the group of fluorescent pseudomonads there are certain denitrifiers, one or more of which are especially adapted to growth in Cohn's solution. Severin and Jensen used the designation *Bacillus pyocyaneus* or *Bacterium pyocyaneum* for their fluorescent denitrifiers; so it is necessary to review the literature relating to the *pyocyaneus* type of organisms.

Pseudomonas aeruginosa (Schroeter, 1886, p. 157) Migula 1900, p. 884 or *Ps. pyocyanea* (Gessard, Flügge)[5] Migula 1895, p. 29. The blue pus organism has long been known but there has been much confusion as to its name. Many writers have used the specific name *pyocyaneus*, altho others have recognized the priority of *aeruginosus*. The question of priority is a difficult one to settle, but the latter term seems to be correct. Bacillus, Bacterium, and

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[3] The formula of this solution is: Distilled water 1000 c. c., KH_2PO_4 5 g., $MgSO_4$ 5 g., neutral ammonium tartrate 10 g., KCl 0.5 g.

[4] The term denitrification in this paper is used strictly to refer to the liberation of free nitrogen from nitrate, not to the reduction of nitrate to nitrite or ammonia.

[5] Gessard (1882) is generally quoted as the author of the term "*pyocyaneus*," altho he did not employ it in accordance with strict taxonomic usage and apparently referred to an entirely different organism. The first correct use of the name *Bacillus pyocyaneus* for the true blue pus organism was by Flügge (1886, p. 286).

Pseudomonas have all three been used as the generic name, according to the generic definitions adopted by different authors.

Gessard (1890) made a comparative study of this organism and some other fluorescent organisms. He concluded that it produces two pigments, a yellow-green, water-soluble pigment, and a blue-green, chloroform-soluble pigment which he called pyocyanin. In this he claims that it differs from *B. fluorescens liquefaciens* and *B. fluorescens putidus* (the non-liquefying type), neither of which produces pyocyanin. Lehmann and Neumann (1896, p. 272), however, claimed that the two organisms differ only in the intensity of the pigment and remark concerning "*B. pyocyaneum*:" "Den Organismus scharf gegen *B. fluorescens* abzugrenzen, geht nach unserer Ueberzeugung nicht an." The opposite conclusion was reached two years later by Niederkorn (1898), who studied a series of fluorescent cultures from various sources and decided that the *fluorescens* type and the *pyocyaneus* type are distinct, altho each has numerous sub-varieties. He states that the flagella of the *pyocyaneus* type are well defined (" wohl ausgeprägte "), but those of the *fluorescens* type are not; that the former takes the Gram stain more definitely than the latter; that the former grows best at 35°, the latter at room temperature. The contrary opinion is expressed by Růžička (1898), who mentions these and other differences (except in regard to the Gram stain), but concludes that they are not constant. By cultivating the *fluorescens* type at 37°, he obtains cultures of the *pyocyaneus* type; by growing the blue pus organism in water, aerated with sterile air, he obtains cultures of the *fluorescens* type. Later Lehmann and Neumann (1912, p. 411–413) continue the discussion, referring to the differences between the two types, laying considerable stress on the denitrifying power of the blue pus organism, but repeating their earlier statement that one type grades imperceptibly into the other. (They do not find either organism Gram-positive.) Finally Pibram and Pulay (1915) made a study of the fluorescent group by serological methods and found it apparently to consist of several different species, *B. pyocyaneum* appearing distinct from *B. fluorescens* altho closely related to it.

The ability of the *pyocyaneus* type to convert nitrate into free nitrogen was apparently first mentioned by Lehmann and Neumann (1896) who do not, however, mention the source from which the culture they studied was obtained. The following year, Weissenberg (1897), apparently at the suggestion of Lehmann or Neumann, made a further investigation of *pyocyaneus* cultures from various sources, finding them all to be denitrifiers, while observing this ability with no organism of the *fluorescens* type. The same year Severin (1897) wrote a paper on denitrifiers obtained from manure, one of which is fluorescent. This fluorescent culture he calls *B. pyocyaneus*, but does not show it to be the cause of blue pus.

One striking fact in this connection is that no one seems to have found a Gram-positive *pyocyaneus* culture which denitrifies or a Gram-negative one which does not denitrify. Those who report denitrification either state the organism to be Gram-negative (as do Lehmann and Neumann) or else make no statement in regard to the Gram stain. Those who have found it to be Gram-positive have not studied its action on nitrate. This suggests that there are two different organisms, one Gram-positive and pathogenic, the other Gram-negative, denitrifying, and probably saprophytic. If this be the case, the former is more likely to be distinct from the *fluorescens* type than the latter.

Pseudomonas putida (Flügge) Migula 1900, p. 912.— The name *Bacillus fluorescens putidus* [6] was given by Flügge to the non-liquefying, fluorescent type of organism. Eisenberg (1891), besides this name, used the name *B. fluorescens non-liquefaciens* for what he considers a different organism, and in this is followed by Kruse (1896) and Migula (1900), the latter discarding the polynomial and renaming it *Ps. Eisenbergi*. Lehmann and Neumann (1912) however, do not consider it a distinct type, and *Ps. putida* is the only non-liquefying species considered today to have good standing.

Whether *Ps. putida* and *Ps. fluorescens* are distinct is also a question that is not entirely settled. Lehmann and Neumann do not question but that they are distinct. Pibram and Pulay (1915), as the result of their serological studies, conclude that they are not only distinct, but that they stand the furthest apart of any of the fluorescent cultures studied. Edson and Carpenter (1912), however, consider that there are so many gradations between rapid liquefiers and non-liquefiers that this characteristic cannot be used to distinguish between species.

THE NUMBER OF FLUORESCENT BACTERIA.

A summary of the literature, therefore, gives no satisfaction in deciding how many different pseudomonads possess the property of producing fluorescence in culture media. Some writers consider them all the same, others make two or three different species, still others believe there are several species, while if we consider every name a distinct species there are a hundred or more. A study of the literature, however, indicates that there are four or five types standing out more or less distinct from each other: 1) *Ps. aeruginosa*, the blue pus organism, a Gram-positive, rapidly liquefying

[6] In the third edition of Flügge's book, Kruse (1896) uses the name *B. fluorescens putridus*, evidently a misprint or mistake in spelling, because Flügge's description of the organism by the term "stinkende" shows plainly that *putidus* was the word he meant to use. Migula in renaming the organism follows Kruse's spelling, calling it *Ps. putrida* (Flügge). Other writers, however, such as Eisenberg, Lehmann and Neumann, and Chester, have used the spelling *putidus*.

organism, producing the blue-green pigment pyocyanin in addition to the fluorescent pigment, and possibly reducing nitrate to nitrogen. 2) *Ps. fluorescens*, a Gram-negative, rapidly liquefying saprophyte, showing poor growth or none at 37°, and unable to convert nitrate to free nitrogen. 3) A Gram-negative, rapidly liquefying denitrifier, such as described by Lehmann and Neumann as *Bacterium pyocyaneum*. Whether these authors worked with the true blue pus organism or not, there seems to be an organism of this description that is different from the true *Ps. aeruginosa*. Several such cultures have been isolated in this laboratory, all of which fail to show pyocyanin even when grown in nitrate broth (the method described by P. Eisenberg [1914, p. 470] as showing the production of this pigment to advantage) and extracted with chloroform. These cultures have differed among themselves and may represent several varieties. Undoubtedly the *B. pyocyaneus* of Severin, Jensen and others who studied denitrifiers from manure and soil was an organism (or organisms) of this type rather than of the true *pyocyaneus* type. 4) A non-liquefying denitrifier described by Van Iterson (1902) as *B. denitrofluorescens*, which is probably distinct from the above and from the following, altho it has not been studied here. 5) *Ps. putida*, a non-liquefying organism, unable to denitrify. Altho some writers seem to think liquefaction an unsatisfactory basis for the separation of these species, there seems no chance for reasonable doubt that an organism unable to liquefy after six months is different from the very rapid liquefiers studied in the present work. The difficulty in making this distinction may perhaps be due to the failure to distinguish between true liquefaction by the living cells and slow digestion of the gelatin by enzymes liberated from the cells after death.

Further investigation is necessary before it can be decided whether these five types represent different varieties of the same species, five separate species, or even five different type species about which distinct groups of species (perhaps genera) should be gathered.

CHARACTERISTICS OF TYPICAL FLUORESCENT ORGANISMS.

Morphology.— Small, short rods, not much over 0.5 micron in diameter or perhaps somewhat smaller; no spores; a few flagella in a tuft at one pole; Gram-negative. (A few fluorescent spore-formers have been described, and Edson and Carpenter (1912) mention a weakly fluorescent peritrichic rod; but these are apparently unrelated organisms. The *pyocyaneus* type has been described as Gram-positive.)

Cultural characteristics.— Growth on agar, smooth, soft to slimy; on potato, smooth, brownish, medium discolored. Nearly all other cultural characteristics vary.

Greenish fluorescence is the most striking characteristic of the entire group, but it is not a constant characteristic. It is produced

in some media and not in others. Gessard (1890, 1892), Lepierre (1895) and Jordan (1899) have studied the ability of these organisms to cause fluorescence, with rather discordant results. They differ considerably in their conclusions as to the composition of the medium necessary for the production of this pigment. The reason for this discrepancy may be in part as suggested by Jordan because of the difficulty in obtaining absolutely pure chemicals; but it is undoubtedly also due to the varying behavior of different varieties. It has been observed in the course of the present work that two different strains may behave exactly opposite so far as concerns their ability to produce fluorescence in one or the other of some two media investigated. Any one strain, moreover, may vary considerably at different times in its ability to produce fluorescence. One particular strain has been studied in this laboratory which was thought to be *Ps. fluorescens* when first obtained from soil, altho not showing fluorescence; but after having been cultivated for several generations on a beef-extract-peptone agar containing 0.1 per ct. nitrate, it gradually became fluorescent, and at the time of writing is one of the most strongly fluorescent cultures in this laboratory. (Another substrain of this organism, kept growing meanwhile on the same agar without nitrate, has developed no fluorescence.) A similar phenomenon was observed by Severin (1897) upon cultivating a denitrifying strain in nitrate broth.

The strain used in Bright's experiments as reported in the first part of this bulletin was always found to cause decided fluorescence on all ordinary media.

Relation to oxygen.— Apparently all of the group are strictly aerobic. This is certainly true of all that have been studied here.

Liquefaction of gelatin.— Typical *Ps. fluorescens* is a very vigorous liquefier. Slow liquefiers are common, as shown by Edson and Carpenter (1912), altho but few have been found in the present work. Non-liquefiers have been observed occasionally in the soils investigated here.

The gelatin colonies of fluorescent organisms vary according to the rapidity of liquefaction. Typical *Ps. fluorescens* produces a rapidly liquefying colony with entire edges, that liquefies the entire plate in a few days. The strain studied in this work produced a colony of this type, also characterized by its clear structureless center; fluorescence was sometimes present, sometimes absent.

Ammonification.— Ammonia is produced from proteid by all the fluorescent organisms so far as they have been studied. Blanchitière (1917) has made a careful study of the ammonification of asparagin by a fluorescent liquefier agreeing well with the strain used in the present work; and has found that it easily converts the amide nitrogen of this compound into ammonia, but the aspartic nitrogen less readily.

Action on sugars and glycerin.— Apparently no fluorescent organism has been recorded as producing gas from sugars or glycerin. Nearly all writers have found acid to be produced from dextrose, but in regard to other sugars the results are conflicting. The reason for this in part, is that the technic generally used is bound to give meaningless results. Thus Tanner (1918) and Edson and Carpenter (1912) both determine acid production by titration, the latter writers titrating hot — a procedure which Clark (1915) has shown to be illogical. Tanner finds acid production only in dextrose, while Edson and Carpenter find it common with the other sugars, undoubtedly because the H-ion concentration is increased by heating the culture previous to titrating.

Blanchitière avoids this error by using litmus agar. He finds acid produced from dextrose and levulose, but not from the disaccharides; but as levulose is a difficult sugar to purify and as Blanchitière says nothing about the source of his sample, he leaves some doubt in the reader's mind as to whether it was actually free from dextrose. He distinctly states that lack of acid reaction in this medium does not mean failure to produce acid, but simply that not enough acid is produced to neutralize the ammonia formed from the peptone. This shows that Blanchitière realizes another source of error but feels unable to overcome it. Plainly, with these two sources of error, the data in the literature as to acid production by fluorescent organisms are not reliable.

In the present work Blanchitière's technic has been modified by using brom cresol purple in place of litmus as an indicator. The result has been in practically every case to find acid produced from dextrose and sucrose but not from lactose and glycerin. The strain studied in Bright's experiments, above reported, showed these reactions very constantly. A synthetic medium containing ammonium tartrate as its only source of nitrogen[7] was then used in an attempt to overcome the error resulting from the presence of peptone in ordinary agar, and somewhat different results were obtained. Even with this method there was no agreement in the results obtained with different fluorescent organisms. The strain used by Bright showed acidity from dextrose and sucrose, the latter reaction disappearing after the first day; while another strain agreeing with it in every other respect showed strong and persistent acidity in sucrose as well as dextrose. The conclusion was reached that *Ps. fluorescens* produces acid from both dextrose and sucrose, but that with the latter sugar the acid production is likely to be obscured by other activities tending to lower the reaction of the medium.

[7] The formula of this medium was: Distilled water 1000 c. c., agar 15 g., $CaCl_2$ 0.5 g., K_2HPO_4 0.5 g., ammonium tartrate 10 g., with 10 g. of the sugar (or glycerin) under investigation.

Reduction of nitrate.— The literature is full of conflicting data in regard to the action of fluorescent bacteria on nitrate. There are several different possibilities: 1) reduction to nitrite; 2) reduction to nitrite, then to ammonia; 3) reduction to ammonia without appreciable accumulation of nitrite; 4) reduction to free nitrogen, i. e. denitrification; 5) assimilation of the nitrogen of the nitrate, with or without previous reduction. It has not proved possible to devise a simple test to distinguish with certainty between these five different possibilities; hence the confusion.

Conversion into free nitrogen is the easiest to determine. We have already seen that fluorescent denitrifiers have been described in the past. Here, they prove to be common enough in soil to be obtained frequently from ordinary soil culture plates. Neither Edson and Carpenter nor Tanner found any among the various cultures they studied; but they both used nitrate broth containing only 0.1 per ct. peptone, in which appreciable gas-production has never been observed here. Most vigorous gas production has been observed in broth or agar containing 1 per ct. peptone. Typical *Ps. fluorescens*, however, has never been found to convert nitrate into nitrogen.

Conversion into ammonia is ordinarily impossible to demonstrate by any simple test, because ammonia can be produced from any nitrogenous substance, and some organic nitrogen is ordinarily present in media. Conversion into nitrate is easy to demonstrate provided the organism investigated does not convert the nitrite into ammonia or assimilate it as fast as produced. *Ps. fluorescens* is generally considered to produce nitrite, but Franzen and Löhmann (1909) studied two strains of what were presumably *Ps. fluorescens* without observing any action at all on the nitrate. Certain strains of fluorescent liquefiers have been studied here which produce no appreciable amount of nitrite in media containing peptone or ammonium chloride, but produce considerable nitrite in an agar containing no nitrogen except potassium nitrate.[8] One strain has been found which does not produce nitrite (or ammonia) even on the latter medium. This suggests that some strains of *Ps. fluorescens* lack the ability to reduce nitrate, wholly or in part, and never attack the nitrate in the presence of more readily available nitrogen. This may explain Franzen and Löhmann's findings.

The question naturally arises whether those that produce no nitrite in ordinary nitrate media constitute a different species from typical *Ps. fluorescens*. So far as tested these differences between the strains have proved constant. Nevertheless the different strains agree in all other particulars investigated, and the data at hand are not considered to warrant the establishment of separate species.

[8] The formula of this medium was: Distilled water 1000 c. c., agar 15 g., $CaCl_2$ 0.5 g., K_2HPO_4 0.5 g., KNO_3 1 g., dextrose or sucrose 10 g.

As typical *Ps. fluorescens* is generally considered to produce nitrite in nitrate broth, the strain selected for Bright's work in the preceding paper was one showing considerable nitrite on all the nitrate media investigated.

Diastatic action on starch.— This test was made by the method of Allen (1918), streaking the cultures over a plate of agar containing soluble starch, and flooding with iodine after seven days. In general no digestion of the starch was observed, altho some of the cultures seemed to show a very narrow zone around the growth where the starch had disappeared.

Action on milk.— Digestion without previous coagulation.

Production of indol.— Statements in the literature are discordant. A number of different strains of *Ps. fluorescens* have been tested here for indol production, a feeble or moderate reaction having been obtained. The test is not considered to have much significance.

BRIEF SUMMARY OF CHARACTERISTICS OF TYPICAL PS. FLUORESCENS.

In the following summary, the characteristics written within parentheses apply to typical cultures only (including the strain studied by Bright); the other characteristics apply not only to typical cultures but to all the cultures studied of the *fluorescens* type (that is type 2, p. 34).

Morphology.— Short, lophotrichic, Gram-negative rods about 0.6 μ in diameter. No spores.

Growth on agar.— Soft, smooth, with greenish fluorescence if conditions are favorable.

Gelatin colonies.— (Large), liquefied, (center structureless), edges entire.

Relation to oxygen.— Strictly aerobic.

Ammonia produced from organic nitrogenous matter.

Acid production.— From dextrose and sucrose, but not from lactose or glycerin.

Nitrates (reduced to nitrite in peptone media) reduced to nitrite in media containing no nitrogen except the nitrate.

Diastatic action on starch.— Weak or none.

Milk.— Digested without coagulation.

PSEUDOMONAS CAUDATUS (WRIGHT 1895, p. 444) CONN.

This organism has been recognized by the writer for a number of years and has been mentioned in previous publications (1913, 1917) but not named. It is now believed to be identical with *Bacillus caudatus* Wright 1895. Earlier surveys of water bacteria (Frankland 1889, Tils 1890, Zimmermann 1890 and 1894) contain descriptions of orange or yellow liquefying bacteria, but they are either meagerly described or else show marked differences from the organism studied here. The identification with Wright's organism is based primarily

upon a colored plate showing the gelatin colony and upon his description of the morphology. He describes the morphology of the organism as follows: "A rather small, slender, non-motile bacillus, with conical ends, occurring often in pairs and in longer forms, sometimes thread-like, which may show irregular segmentation; no spore formation observed." His illustration of the colony agrees well with the present organism as to structure, and agrees as nearly with the shade of orange observed as could be expected in a colored plate twenty years old. Altho Wright does not give the size of the organism in exact figures and calls it immotile, there is little question as to its identity.

Morphology.— Ordinarily the organism is a very slender rod, so small that its diameter is difficult to measure with the ordinary microscope. It is about 0.2 micron in diameter. Its length is ordinarily about 2 microns, but as stated by Wright, longer forms occur. These rods stain solid with the ordinary bacterial stains, such as fuchsin or methylen blue; but with the more delicate dye, rose bengal, they appear to be made up of tiny granules. Cultures a few days old are sometimes made up wholly of these granules, each about 0.2 micron in diameter. Such a preparation looks like a very tiny micrococcus. Cultures of this sort have proved to be alive upon transfer to fresh media; but whether the granules are capable of growth or whether the multiplication is carried on by a few stray rods present in too small numbers to be observed under the microscope is still an unanswered question. This suggests very strongly Löhnis and Smith's idea (Löhnis and Smith 1916) as to life cycles among bacteria, but as yet it has not proved possible to find whether that is the true explanation in this case. The granules may be degenerate forms, a possibility suggested by the rapidity with which cultures die, or the organism may actually be a coccus that has a tendency to produce short chains or filaments in young cultures.

The majority of the cultures show no motility, altho occasionally one is observed that is distinctly motile. This undoubtedly explains why Wright called the organism immotile. An idea of the difficulty in studying motility can be obtained from the trouble encountered in demonstrating flagella on the strain used in Bright's work. This strain was kept under observation for a few months without observing any motility, when at last, quite unexpectedly, a distinctly motile culture of it was obtained. On the same day, two other strains, previously showing no motility, were found to be motile. No apparent reason could be found for this sudden development of motility, which persisted thru at least three or four generations. Meanwhile flagella preparations were made from the strain used by Bright in the work reported above, and one or two organisms were observed with a single flagellum each. This flagellum is

rather long in comparison to the length of the rod. This finding agrees with previous studies of this organism made by the writer. Four strains, in all, have been successfully stained, and about twenty different organisms have been observed with a single polar flagellum each. Preparations were always too poor to allow photomicrographs, but there seems to be sufficient evidence to establish the presence of one polar flagellum. For this reason, Wright's name *Bacillus caudatus* is changed to *Pseudomonas caudatus*.

Chromogenesis.— Next to its morphology, pigment-production is the most striking characteristic of *Ps. caudatus*. The pigment grades from yellow to orange. On potato and gelatin it is generally distinctly orange, while on beef-extract-peptone agar it is more of a yellow. Its color on the latter medium is practically the same as that which is typical of the orange pyogenic cocci, designated cadmium orange by Winslow and Winslow (1908) in their book on the coccaceae. The typical color, indeed, is exactly the shade of cadmium orange which the Winslows found most common among the orange cocci. One strain has been found which was typical in color upon isolation from soil, but which lost its chromogenesis upon cultivation, not regaining it even after cultivating for a while in sterilized soil. This strain retained its typical morphology and differed from the other cultures at first in no other respect except that it was unable to digest soluble starch. Later it was found to have lost its power of producing nitrite upon nitrate-peptone media. No data are at hand to show whether or not it digested starch before it lost its pigment-producing power. The change in the color of this culture can hardly have been due to an impurity, because three separate substrains of this one strain all lost their pigment-producing power at exactly the same time. This shows that chromogenesis, striking as it is in typical cultures, is not an absolutely constant characteristic.

Physiology.—Perhaps the most striking physiological peculiarity of the organism is the difficulty of cultivating it under laboratory conditions. The only way found to keep it vigorous is by transfers every few days onto agar that has been freshly melted and solidified so as to have considerable water of condensation on its surface. This fact is unfortunate, for it makes it practically impossible to keep stock cultures of the organism for purposes of comparison with cultures of other investigators.

Relation to oxygen.— The organism is very strictly aerobic. In fact, it grows poorly in liquid media, even in an open test-tube.

Liquefaction of gelatin.— All cultures liquefy gelatin. The rapidity of liquefaction varies, altho in general it is quite rapid.

Gelatin colonies usually liquefy to a diameter of about 1 cm. in four days. Liquefaction is most rapid on the plates made directly from soil, old cultures liquefying more slowly. The colonies have

typically a radiate structure, altho the typical structure is observed only immediately after isolation from soil. Edges of colonies are entire.

Ammonia production.— As shown by Bright in the preceding paper, *Ps. caudatus* is a vigorous ammonifier.

Action on sugars and glycerin.— In the early work with this organism (Conn, 1913, 1917), tests for acid production were made in sugar broth as recommended in the report of the Committee on Water Analysis of the American Public Health Association. Very irregular results were obtained and in mentioning this type (1913, p. 103) question marks were placed over those figures in the group number referring to dextrose, sucrose and glycerin, altho at that time no evidence at all of acid-production in lactose had been obtained. Later (1917, p. 8) it was thought that this irregularity must be due to poor growth in liquid media, so the recent tests have been made in sugar agar containing some indicator. The most satisfactory indicator has proved to be brom cresol purple. Using standard agar in this work, the strains studied were divided into two groups, one [9] producing no acid and the other (containing the majority of the strains) producing acid from dextrose and sucrose but not from lactose or glycerin. There proved to be some irregularity upon repetition of the test, but not a great deal. It was then felt that the difference between these two groups of strains might be that one produced more alkalinity from the peptone than the other and that its acid-production was thus obscured. The test was therefore repeated a few times on a tartrate agar [10] in which *Ps. caudatus* was found to cause no change in reaction unless some sugar were present. With this medium more consistency was observed upon repetition of the test, but the difference between the two groups was still sharp. The acid group acidified lactose in this medium as well as dextrose and sucrose. It is therefore concluded that typical *Ps. caudatus* produces acid from dextrose, sucrose, and lactose, but not from glycerin, its acid-production from lactose being too weak to neutralize the alkinity produced from the peptone if growing in ordinary media. The non-acid strains (with the exception of the non-chromogenic one) died while under cultivation in the laboratory; so it is felt that their failure to produce acid may have been the first evidence of loss of vigor. Hence they are not considered to be distinct from the typical acid-formers. The strain used by Bright in the experiments reported above was a vigorous acid-producer.

Nitrate reduction.— Irregular results were obtained with this test also. Ordinary nitrate broth proved so unsatisfactory that tests

[9] One of the strains in this group was the one that had lost its pigment producing power.

[10] Formula given in footnote [7].

were made on agar slants as described for *Ps. fluorescens* p. 37. On beef-extract-peptone agar, the acid group of strains, above mentioned, showed a strong nitrite reaction; the non-acid group, with the exception of the non-chromogenic strain, showed no nitrite; the non-chromogenic strain, when first tested, was distinctly nitrite-positive, but after a few months all three substrains of this organism were found to have lost their nitrite-producing power. To investigate this matter further, the synthetic sucrose-nitrate agar [11] already used for *Ps. fluorescens* was employed. On this agar an occasional culture was found to produce nitrite that had showed no nitrite-reaction on the nitrate-peptone agar, and ammonia was observed in almost all cases. Growth was very poor, however, with the non-acid group of strains. Inasmuch as there was no possible source of ammonia in this medium except the nitrate, the conclusion was drawn that *Ps. caudatus* reduces nitrate to nitrite and ammonia, but that some cultures convert the nitrite into ammonia so rapidly that a nitrite test is generally negative. The presence or absence of the nitrite test depends upon the relative rate of these two processes, which is probably associated with the vigor of the culture. Hence the failure of the nitrite test is no proof that any particular culture is not *Ps. caudatus*.

Diastatic action on starch.— This test was made by the method of Allen (1918). All the cultures of *Ps. caudatus* studied, except the non-chromogenic strain, gave a strong reaction, but the non-chromogenic strain showed no digestion of the starch. These results were the same upon frequent repetition of the test.

Action on milk.— No change in appearance or reaction.

Production of indol.— The results of this test have generally been negative, altho a few cultures have shown a weak reaction. It is not impossible that they would all produce indol if tested under conditions favorable to the growth of this organism; but the test has always been made in liquid media (Dunham's solution) and as yet no effort has been made to improve the technic.

BRIEF SUMMARY OF CHARACTERISTICS OF TYPICAL PS. CAUDATUS.

In the following summary, the characteristics written within parentheses apply to typical cultures only (including the strain studied by Bright); the other characteristics apply to all the strains studied:

Morphology.— Long, slender, granular, Gram-negative rods, about 0.2μ in diameter, with a single polar flagellum. No spores. Old cultures often appear like cocci, $0.2–0.4\ \mu$ in diameter.

Growth on agar.— Soft, smooth (cadmium orange).

[11] Formula given in footnote 8. Sucrose (not dextrose) was used in this formula because of the presence of ammoniacal impurities in the dextrose on hand.

Gelatin colonies.— Small (to medium sized) — i. e. up to 1 cm. in diameter, (orange, structure radiate), edge entire.

Relation to oxygen.— Strictly aerobic.

Ammonia produced from organic nitrogenous matter.

Acid production.—(From dextrose, sucrose, and lactose) but not from glycerin.

Nitrates reduced to nitrite and ammonia (with accumulation of nitrite).

Diastitic action on starch.— (Strong).

Milk unchanged.

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THE USE OF THE NITRATE-REDUCTION TEST IN CHARACTERIZING BACTERIA.*

H. J. CONN AND R. S. BREED.

SUMMARY

1. The diagnostic value of the nitrate-reduction test has been studied with various media and under various conditions. Four different species (or groups of species) were used: the colon group, *B. cereus* Frankland, *Ps. fluorescens* (Flügge) Migula, and *Ps. caudatus* (Wright) Conn.

2. Three important sources of error were found: poor growth on the media used; failure of the organism investigated to reduce nitrate in the presence of more readily available nitrogen; reduction to ammonia or free nitrogen without accumulation of nitrite. Negative results are often due to one of these sources of error; but it is often difficult or impossible to devise conditions for making the test which eliminate these errors.

3. A negative nitrite test is meaningless until these sources of error have been eliminated. Nitrate reduction on any medium or under any condition indicates that an organism is a nitrate-reducer, even tho negative results are secured with the ordinary tests. Hence no organism can be safely called a non-nitrate-reducer until exhaustive tests have been made. Such tests are not adapted to routine bacteriological investigations.

INTRODUCTION.

For some time bacteriologists have used the ability to reduce nitrates as a criterion for distinguishing certain kinds of bacteria. The test has been regarded as of so much diagnostic value that it has been included in nearly all schemes of bacterial characterization, among the most important of which is the descriptive chart adopted by the Society of American Bacteriologists. An investigation of this test was begun as a result of some student work done under the direction of one of us (B) upon certain cultures of the colon group. Such very irregular results were obtained in regard to the power of reducing nitrates that the method of making the

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test was decided to be unsatisfactory. Further work was therefore undertaken, at first by students and volunteer assistants, and then by one of us (C) as a contribution to the work of the Committee on the Descriptive Chart appointed by the Society of American Bacteriologists. For the earlier part of the work much credit is due to Emma Edson Breed, H. M. Weeter, and H. V. Grant.

TECHNIC.

1. *Formula of medium.*— An attempt was made at first to follow the "standard" technic adopted by the American Public Health Association. It was soon found, however, that a standard technic was sadly lacking. In the 1905 Report of the Committee on Standard Methods of Water Analysis[1] the following directions are given: "Dissolve one gram peptone in one liter of tap water, and add two grams of nitrite-free potassium nitrate. It is convenient to prepare a stock solution of potassium nitrate by dissolving four grams of solid nitrate in 100 c.c. of distilled water and use five c.c. of this solution in the above formula." A little arithmetic will show that to follow the direction given in the first sentence would give a 0.2 per ct. solution, that in the second sentence would give a 0.02 per ct. solution of the nitrate. In the 1912 report[2] an attempt was evidently made to correct this disagreement, because the two grams in the first sentence was changed to 0.2 g.; but by some slip the four grams in the second sentence was also changed to 0.4 g., thus giving a 0.002 per ct. solution. With this inconsistency there has naturally been a great variation in the "standard" nitrate broths used by different bacteriologists. This is shown by a survey of the literature. Thus Gorham[3] recommends 1 g. of peptone and 0.2 g. of potassium nitrate (i.e., 0.02 per ct.); while Chester[4] recommends a formula different from any of the above — 10 g. of peptone and 0.02 g. (i.e., 0.002 per ct.) of *sodium* nitrate. Apparently in preparing a formula for nitrate broth the figures 1 for the peptone and 2 for the nitrate have been considered more important than the position of the decimal point in either case. This peculiar irregularity has already been mentioned by one of us.[5]

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[1] Report of Committee on Standard Methods of Water Analysis. *Jour. Infec. Diseases*, Suppl. 1: 1–127. 1905. See p. 109.

[2] Standard methods for the examination of water and sewage. Second edition, 144 pp., New York, 1912. See p. 135.

[3] Gorham, F. P. Laboratory course in bacteriology, 192 pp., Philadelphia, 1901. See p. 92.

[4] Chester, F. D. Manual of Determinative Bacteriology, vi+401 pp., New York, 1901. See p. 34.

[5] Breed, R. S. The standard method of determining nitrate reduction. Paper read at Philadelphia meeting of Soc. of Amer. Bacteriologists, Abs. in *Sci.*, N.S., 41:661. 1915.

In the early part of this work two different nitrate broths were used independently by two of the workers, each thinking he was following the standard technic; both contained 0.1 per ct. peptone but one contained 0.2 per ct. KNO_3 and the other 0.02 per ct. KNO_3. When the discrepancy was noticed, the two media were compared, as were other variations of nitrate media, all of them within the limits of variation of the formulae given above. Later, other arbitrary variations were made for special purposes, as will be explained later.

2. *The nitrite test.*— Two different variations of the official nitrite test were used in the first part of this work, differing only in the quantity of the reagents used. Each variation was supposed to be " standard " by the particular investigator employing it, owing to two different interpretations of the standard methods. On page 120 of the report for 1912[6] the following technic is given: " Remove three c. c. of the culture to a clean test tube and add two c. c. of each of the naphthylamine solution and the sulphanilic acid solutions described under the determination of nitrites (see page 22)." Three of the workers followed these directions exactly. Another, working independently, looked up page 22 and found that at this place the directions were to add 2 c. c. to 100 c. c. of the water to be tested, and concluded that the proportions of reagents given on page 120 must have been a mistake; so he used the reagents in the proportion of about 2 parts to 100 parts of the medium, i. e. a few drops to the culture tube. Later these two methods of using the reagents were compared, and the latter method was found to be the better. Using larger quantities of the reagent was found to dilute the nitrite present to such an extent as to obscure the reaction in cases where only small amounts of nitrite had been formed, especially when the reagents were old and somewhat discolored. As a result, in the latter part of the work, only a few drops of each reagent were added to the culture.

A still different test was used in some of the early part of the work: the potassium-iodide-starch test, as described by Erwin Smith[7] This test compared very favorably with the official test, but proved a little less delicate and the reagents were found to deteriorate more rapidly; so its use was discontinued. This test is preferred by Harding[8] just because it is less delicate; but as the present work progressed, the need of a delicate nitrite test was emphasized more and more.

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[6] See footnote 2.

[7] Smith, Erwin. Bacteria in relation to plant diseases. Carnegie Inst., Washington, Pub. 27, Vol. I. 1905. See p. 63.

[8] Harding, H. A. The constancy of certain physiological characters in the classification of bacteria. N. Y. Agr. Exp. Sta., Tech. Bul. 13, 1910. See p. 34.

WORK WITH THE COLON GROUP.

In 1912, one of the students taking part in the work (E. E. B.)[9] observed considerable irregularity in the nitrate-reduction test in the case of fifty different cultures of the colon group isolated from polluted water. These cultures included all four of the commonly recognized types, as distinguished by their fermentative reactions with sucrose and dulcite. They were tested in triplicate in 0.02 per ct. nitrate broth (with 0.1 per ct. peptone), some giving negative results in all three tubes, others giving inconsistent results, while the majority of the cultures gave consistent positive results as was expected. In 1913 the cultures giving no positive reactions the first time tested were tested again by the same student, using the same medium, and all but three of the cultures were found to produce nitrite in at least one out of three tubes. These three cultures and one other (which had shown nitrite in only one tube each time tested) were tested a third time with consistently positive results. These findings are listed in Table I under the heading " Series of 1912–13." In all, about 64 per ct. of the tests were positive, counting each tube in a set of triplicates as a single test. The fact that every culture gave a positive reaction in the end suggested that all might be nitrate-reducers, but that the methods of testing were such that they did not develop this ability in more than about two-thirds of the cases.

Later in 1913 (see Table I, series of 1913) the same cultures were tested in triplicate again by another student (H. M. W.) using the same medium and the same methods generally, recording the results similarly except that a faint nitrite reaction was denoted " trace " (" T " in Table I) instead of " +." By this student 37 cultures were tested twice, 15 three times. Until the formula of the medium was varied, only about 37 per ct. of the tests were positive. In regard to the individual cultures, there was practically no agreement with the results of the first student: only three cultures (Nos. 11, 14 and 35) gave consistently positive reactions in the hands of both students; not one gave consistently negative reactions on both occasions, while nearly all the cultures that gave irregular results in the hands of the first student did so with the second student also.

In the course of this work it was noticed that no strain grew well in the medium used; so some preliminary work was done in varying the composition of the medium (last column under " Series of 1913," Table I). Twelve cultures that had given negative reactions in the majority of cases were tested again in a medium containing 0.2 per ct. (instead of 0.1 per ct.) peptone. The growth in this medium was noticeably better, and a distinct nitrite reaction was obtained in all 36 tubes.

[9] Work done at the Allegheny College Biological Laboratory.

TABLE I. NITRATE REDUCTION BY ORGANISMS OF THE COLON GROUP IN 0.02 PER CENT NITRATE BROTH.

Tests made in triplicate; + and — indicate presence and absence respectively of a distinct nitrite reaction in all three tubes; ± indicates a distinct nitrite reaction in two out of the three tubes, ∓ in only one of the three tubes. T indicates mere trace of nitrite.

Culture No.	Series of 1912–13.			Series of 1913.				Series of 1914.		
	Peptone 0.1%.			Peptone 0.1%.			Peptone 0.2%.*	Peptone 0.1%.	Peptone 0.2%.	Peptone 0.5%.
	1st test.	2nd test.	3rd test.	1st test.	2nd test.	3rd test.				
1	—	+	......	—	—	+	......	......	......	......
2	—	∓	......	—	∓	......	......	∓	+	+
3	+	......	......	—	+	......	......	∓	+	+
4	±	+	......	—	±	......	......	—	+	+
5	—	+	......	—	+†	......	......	±	+	+
6	±	±	......	—	T	+	......	—	+	+
7	+	......	......	—	—	+	+	+	+	+
8	—	+	......	±	......	......	......	—	+	+
9	+	......	......	—	T‡	......	......	—	+	+
10	—	......	......	—	∓	......	......	±	+	+
11	+	......	......	+	+	......	......	+	+	+
12	±	......	......	—	—	+	......	∓	+	+
13	+	......	......	—	±	......	......	+	+	+
14	+	......	......	T	+	......	......	......	......	......
15	+	......	......	±	......	......	......	+	+	+
16	T?	+	......	—	T	—	+	+	+	+
17	—	+	......	—	+	......	......	......	......	......
18	±	......	......	±	......	......	......	—	+	+
19	+	......	......	—	±†	......	......	—	+	+
20	—	+	......	—	∓	—	+	—	+	+
21	∓	+	......	+	......	......	......	—	+	+
22	—	+	......	—	∓	......	......	+	+	+
23	+	......	......	—	T	+	......	±	+	+
24	+	......	......	—	±	......	......	—	+	+
25	—	+	......	—	+†	......	......	+	+	+
26	—	+	......	—	—	+	+	......	......	......
27	∓	+	......	—	+	......	......	—	+	+
28	+	......	......	±	......	......	......	∓	+	+
29	+	......	......	—	—†	+	+	∓	+	+
30	+	......	......	—	∓	......	......	±	±	+
31	—	+	......	—	—	±	+	—†	+	+
32	+	......	......	∓	+	......	......	—	+	+
33	+	......	......	—	......	......	......	—	±†	+

* This medium contained only 0.01 per ct. of nitrate.
† One of the tubes showed nitrite present in mere traces only.
‡ One of the tubes showed a distinct positive reaction.

TABLE I—(*Concluded*).

Culture No.	Series of 1912–13.			Series of 1913.				Series of 1914.		
	Peptone 0.1%			Peptone 0.1%			Peptone 0.2%.*	Peptone 0.1%.	Peptone 0.2%.	Peptone 0.5%.
	1st test.	2nd test.	3rd test.	1st test.	2nd test.	3rd test.				
34	—	+	......	—	+	+	+	±	+	+
35	+	......	......	+	......	......	......	+	+	+
36	+	......	......	—	+	......	......	+	+	+
37	+	......	......	—	—	+	+	∓	+	+
38	—	+	......	—	—	—	+	±	+	+
39	+	......	......	—	—	+	+	—	+	+
40	±	......	......	—	—	......	......	—	+	+
41	—	—	+	+	±†	......	......	+	+	+
42	......	......	......	—	T	—	+	—	+	+
43	—	—	+	—	+	......	......	—	+	+
44	+	......	......	—	+	+	+	+	+	+
45	—	—	+	+	—	?	+	±	+	+
46	∓	∓	+	+	......	......	......	—	+	+
47	+	......	......	∓	T	......	......	—	+	+
48	+	......	......	+	±	......	......	±	+	+

* This medium contained only 0.01 per ct. nitrate.
† One of the tubes showed nitrite present in mere traces only.

The idea suggested by the last mentioned tests was followed up by a third student (H. V. G.) the next year. Parallel tests were made in nitrate broth containing 0.1, 0.2, and 0.5 per ct. peptone respectively (Series of 1914, Table I). With 0.1 per ct. peptone only 42 per ct. of positive results were obtained, and the agreement of the individual cultures with their previous behavior was no greater than it had proved to be the preceding year. With 0.2 per ct. peptone 98.5 per ct. of the tests (129 out of 131 tubes) gave positive results, every culture showing a positive reaction in at least two of the tubes. With 0.5 per ct. peptone all of the cultures gave positive reactions in all three of the triplicate tubes. Further tests not listed in the table were made with a medium containing 0.1 per ct. peptone and 0.2 per ct. (instead of 0.02 per ct.) nitrate. This use of ten times the original amount of nitrate was found to have no influence on the results, the irregularity proving as great as with 0.1 per ct. peptone and 0.02 per ct. nitrate.

The conclusion reached by this work was that inconsistent results of the nitrate-reduction test may be expected with organisms of the

colon group unless enough peptone is present to furnish these bacteria with conditions favorable to their vigorous growth. With 0.1 per ct. peptone the growth was generally poor; with 0.2 per ct. fairly good; and with 0.5 per ct. very good. The amount of nitrate present apparently had little influence on the results. The important matter was vigorous growth of the organisms; and under conditions allowing vigorous growth all the cultures of the colon group tested proved to reduce nitrate to nitrite.

WORK WITH BACILLUS CEREUS FRANKLAND.

As an organism to contrast with those of the colon group, *B. cereus* Frankland was chosen.[10] *B. cereus* grows well in the presence of considerable organic matter, but it does not seem to require the large amounts of nitrogenous material that the colon organisms do. The cultures of *B. cereus* used were isolated by one of us (C) from soil.

Ten out of 130 cultures, apparently all *B. cereus*, failed to produce nitrite when tested promptly after isolation from soil in broth containing 0.1 per ct. peptone and 0.2 per ct. KNO_3. One of these ten cultures (which we will denote Culture x) was tested again two years later (Table II, Test No. 2) together with seven typical *B. cereus* cultures (which we will call Cultures A to G respectively) that had produced nitrite the first time tested. This work was done by a different investigator (H. V. G.) from the one who made the original tests. In order to see whether the explanation for the disagreement previously found might be due to the same cause as the disagreement in the case of the colon organisms, the four following nitrate broths were used:

0.1 per ct. peptone, 0.2 per ct. nitrate;
0.1 per ct. peptone, 0.02 per ct. nitrate;
0.2 per ct. peptone, 0.02 per ct. nitrate;
0.5 per ct. peptone, 0.02 per ct. nitrate.

Each culture was inoculated in triplicate into each medium; and clear-cut positive nitrite reactions were obtained in all cases, even with Culture x.

A few months later (Test 3, Table II) Cultures A and B, together with seven other typical nitrite-positive *B. cereus* cultures,[11] were tested again in the first of the nitrate broths listed above, all giving such clear-cut positive reactions that no further investigation of

[10] This organism was identified by means of the characteristics previously described.— Conn, H. J., The spore-forming bacteria in soil. N. Y. Agr. Exp. Sta., Tech. Bul. 58. 1917.

[11] Not listed in Table II, because no further work was done with these seven cultures.

these cultures was made. At the same time, six other cultures were retested in this same medium, and also in media of the following composition:

0.5 per ct. peptone, 0.2 per ct. nitrate;
0.5 per ct. peptone, 1 per ct. dextrose; 0.2 per ct. nitrate.

One of these six cultures (Culture X, Table II) had given a positive reaction the first time tested, while the others (Cultures a, b, c, d, and x) had given negative reactions. The results showed no nitrite from Cultures a, b, c, d, and none from Culture X, while nitrite was found with Culture x, as had been the case in Test 2. In other words all the Cultures except X and x showed agreement with the original test; but X had changed from nitrite-positive to nitrite-negative, x from nitrite-negative to nitrite-positive.

Test No. 4 was made a few weeks later. This test included Cultures b, c, x, and X, together with a fifth, which from its similarity to Culture X will be denoted as Culture Y. Cultures b, c, x, and X gave the same results as in Test No. 3. Culture Y, like Culture X, had given a positive nitrite reaction at the time of isolation two years earlier, but now showed no production of nitrite.

No further tests of Culture Y were made, but Cultures X, a, b, c, d, and x were tested again about a month later (Test No. 5). This test was made in six different nitrate broths, the four used in Test No. 2, together with two additional broths as follows:

0.2 per ct. peptone, 0.2 per ct. nitrate;
0.5 per ct. peptone, 0.2 per ct. nitrate.

All the cultures except Culture c gave the same results as in the two preceding tests, no difference being observed between their behavior in the different media. Culture c gave distinctly negative results with the four media used in Test No. 2; but with the two new media (that is, the media with 0.2 per ct. nitrate combined with more than 0.1 per ct. peptone) a discrepancy was observed on the 10th day, one of the two duplicate tubes of each medium tested giving a positive nitrite test.

Four years later Culture a was tested again, still with negative results. In the meanwhile the advantage of agar for making the test in doubtful cases (see p. 11) had been learned, and so this culture at this time was tested also on beef-extract-peptone agar to which 0.1 per ct. KNO_3 had been added. The results were still negative. Good growth, however, was obtained in all cases.

Test No. 7 was made a few months later. Three organisms were tested this time: H, a typical nitrite-positive *B. cereus* culture that had not been tested before since immediately after isolation five years before; Culture X, and Culture b. These cultures were tested at

TABLE II. NITRITE PRODUCTION BY *B. cereus* IN VARIOUS NITRATE MEDIA.

Tests made in triplicate. Each + or — sign indicates presence or absence of nitrite, in all three tubes of one set. The sign ± indicates disagreement among three parallel tubes.

	ORIGINAL TEST, 1911–13.	SECOND TEST, 1914.				THIRD TEST, 1914.			FOURTH TEST, 1914.
Medium containing { KNO₃ / Peptone	0.2% 0.1%	0.2% 0.1%	0.02% 0.1%	0.02% 0.2%	0.02% 0.5%	0.2% 0.1%	0.2% 0.5%	*0.2% 0.5%	0.2% 0.1%
Culture A	+	+	+	+	+	+	.....	.....	.....
Culture B	+	+	+	+	+	+	.....	.....	.....
Culture C	+	+	+	+	+	.....	.....	.....	.....
Culture D	+	+	+	+	+	.....	.....	.....	.....
Culture E	+	+	+	+	+	.....	.....	.....	.....
Culture F	+	+	+	+	+	.....	.....	.....	.....
Culture G	+	+	+	+	+	.....	.....	.....	.....
Culture H	+	.....	.....	.....	.....	.....	.....	.....	.....
Culture X	+	.....	.....	.....	.....	—	—	—	—
Culture Y	+	.....	.....	.....	.....	.....	.....	.....	—
Culture a	—	.....	.....	.....	.....	—	—	—	.....
Culture b	—	.....	.....	.....	.....	—	—	—	—
Culture c	—	.....	.....	.....	.....	—	—	—	—
Culture d	—	.....	.....	.....	.....	—	—	—	.....
Culture x	—	+	+	+	+	+	+	+	+

	FIFTH TEST, 1915.						SIXTH TEST, 1918.		SEVENTH TEST, 1918.	
Medium containing { KNO₃ / Peptone	0.2% 0.1%	0.2% 0.2%	0.2% 0.5%	0.02% 0.1%	0.02% 0.2%	0.02% 0.5%	0.2% 0.1%	†0.1% 1.0%	†0.1% 1.0%	‡0.1% 0.0%
Culture A	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Culture B	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Culture C	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Culture D	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Culture E	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Culture F	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Culture G	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Culture H	.....	.....	.....	.....	.....	.....	.....	.....	+	+
Culture X	—	—	—	—	—	—	.....	.....	—	—
Culture Y	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Culture a	—	—	—	—	—	—	—	—	.....	.....
Culture b	—	—	—	—	—	—	.....	.....	—	—
Culture c	—	±	±	—	—	—	.....	.....	.....	.....
Culture d	—	—	—	—	—	—	.....	.....	.....	.....
Culture x	+	+	+	+	+	+	.....	.....	.....	.....

* This medium contained 1 per ct. dextrose.
† Nitrate-peptone agar. Test made on agar slants.
‡ Five different nitrate agars used, containing no added organic matter except sugar. Tests made on agar slants. Each medium inoculated in triplicate and the three tubes tested on different days. Each + or — sign indicates presence or absence of nitrite in all fifteen tubes.

this time in six different agar media, all but one of them containing no added organic matter except some sugar. Culture H gave positive results in all the media used; Cultures X and b gave negative results thruout, altho one tube out of nine in the case of Culture b gave a positive nitrite reaction on the 7th day (due probably to an impurity). One of the media used contained no possible source of ammonia except the nitrate, so a Nessler test was made to see if the nitrate had been converted into ammonia without accumulation of nitrite. This test also was negative.

As a result of this work it was concluded that with *B. cereus* the explanation of the irregularities is not as simple as in the case of the colon organisms. *B. cereus* grows well in almost any medium and ordinarily reduces nitrate to nitrite. Certain cultures, however, seem to lack this reducing power, either temporarily or permanently, altho they grow well in the media used. In general, constantly negative or constantly positive results have been found with any particular culture. This suggests that there may be two different species so closely related that they can be distinguished only by means of the nitrate test. Three cultures (X, Y, and x), however, and possibly a fourth (c), gave inconsistent results. Altho this disagreement may possibly have been due to a contamination that ran out the original organism, the agreement in all other respects with the original descriptions makes this explanation doubtful. No other explanation of the irregularity has been found, however, unless it be assumed that cultures of *B. cereus* may lose or gain the power of reducing nitrates when cultivated in the laboratory.

WORK WITH FLUORESCENT PSEUDOMONADS.

During the course of a study of soil bacteria, numerous cultures of fluorescent pseudomonads were obtained that appeared to be closely related altho differing in certain particulars. One of the most noticeable points of difference was that some produced nitrite in nitrate broth containing 0.1 per ct. peptone while others produced no nitrite under these conditions. With these organisms the growth was always fairly good; nevertheless it was felt that the difference might be due to causes similar to those affecting the nitrate-reduction by the colon organisms. Upon testing the cultures in broth containing larger amounts of peptone (as much as 1 per ct.) the same distinction between nitrite-producers and non-nitrite-producers held true; but in this broth a further difference appeared, some of the organisms producing gas (presumably free N), others producing no gas. In other words three groups of fluorescent pseudomonads were found upon inoculation into 1 per ct. peptone solution containing nitrate: 1) producing nitrite and gas; 2) producing nitrite

but no gas; 3) producing neither nitrite nor gas (in appreciable quantities).

As the fluorescent organisms grow rather better on the surface of agar than in a liquid medium, further work was carried on in a nitrate agar containing beef-extract 0.3 per ct., peptone 1 per ct., KNO_3 0.1 per ct. The nitrite test was made by pouring the reagents upon the surface of the agar slant after incubation; gas was detected by means of bubbles and cracks in the medium. One day of incubation was enough to bring out the nitrite reaction with vigorous nitrate-reducers; but cultures were generally kept until the 7th day. This agar slant test was found to give the same results as obtained with the 1 per ct. peptone broth cultures; the same three groups were found as before.

For further testing six cultures were selected, two of each group. These six cultures will be denoted in this paper as follows:

AA, BB } Producing nitrite and gas in nitrate-peptone media.

A, B } Producing nitrite without gas in nitrate-peptone media.

a, b } Producing neither nitrite nor gas in nitrate-peptone media.

These six cultures were retested not only on the nitrate-peptone agar previously used (Columns 2 and 3, Table IV) but also on agar containing no nitrogen (disregarding impurities) except 0.1 per ct. KNO_3 and with no added organic matter except sugar (Columns 4 to 17, Table IV). The object was to learn whether the nitrate was converted to ammonia. The reports of the Committee on Water Analysis[12] have called attention to the need of making the ammonia test, but have added that ammonia may also come from the peptone. Kligler[13] emphasized the importance of this source of error. As the fluorescent organisms are all ammonifiers, it is impossible to tell whether the ammonia present in a nitrate-peptone medium comes from the nitrate or the peptone. Hence it was decided to grow them under conditions where there could be no source of ammonia except the nitrate, so that its presence would show reduction of the nitrate even tho there were no accumulation of nitrite. The first media tried contained dextrose (Media DM, and DL, Table III) and were found to be unsatisfactory for the ammonia test because no dextrose could be obtained free from ammonia. Lactose was found to have the same disadvantage, so finally sucrose (Medium S, Table III) was employed as a source of energy.

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[12] P. 115 of reference given in footnote 1, p. 120 of reference given in footnote 2.

[13] Kligler, I. J. The source of ammonia and nitrites in a nitrate-peptone solution. *Amer. Jour. Pub. Health*, 8:788–792. 1913.

TABLE III. COMPOSITION OF NITRATE MEDIA USED IN WORK WITH *Ps. fluorescens* AND *Ps. caudatus*.

Figures indicate grams per litre.

Ingredients.	MEDIA SYMBOLS.†					
	P	DM	D	DL	S	DA
Agar*	15.	15.	15.	15.	15.	15.
Peptone	10.	....	....	....	....	....
Dextrose	....	10.	10.	10.	....	10.
Lactose	....	....	....	5.	....	....
Sucrose	....	....	....	....	10.	....
KNO_3	1.	1.	1.	1.	1.	1.
$CaCl_2$	....	0.5	0.5	0.5	0.5	0.5
$MgSO_4$	....	5.	....	....	....	....
K_2HPO_4	....	5.	0.5	0.5	0.5	0.5
NH_4Cl	....	....	....	....	....	2
Beef extract	3.	....	....	....	....	....

* "Bacto-agar" (a purified agar sold by the Digestive Ferments Co.) was used in all except Medium P.

† In these media symbols, the letters represent the significant ingredients, as follows: P, peptone; D, dextrose; M, magnesium sulphate; L, lactose; S, sucrose; A, ammonium chloride.

Even before a satisfactory ammonia-free medium was obtained, some very interesting results were procured from the use of these media (see Table IV). On these media all six cultures, even including Strains a and b, were found to give the nitrite test, sometimes in mere traces, but often in appreciable quantities, as early as 24 hours after inoculation. The test on the ammonia-free Medium S (Table IV) gave the following results: Cultures AA and BB showed the presence of ammonia, as well as the nitrite and gas demonstrated on the peptone media; Cultures A and B showed a very strong nitrite reaction, but a weak ammonia reaction after the fourth day; Cultures a and b showed a moderate nitrite reaction and an ammonia reaction slightly stronger than with Cultures A and B. The ammonia reactions were in no case strong enough to prove that the organisms were converting the nitrate rapidly into ammonia.

A further test (last two columns, Table IV) was then made on a synthetic agar (Medium DA, Table III) containing nitrate, dextrose, and ammonium chloride, and very interesting results were obtained. Cultures A, B, a, and b gave the same reactions (i. e. either nitrite-positive or nitrite-negative) as on peptone media; of the gas-producers, BB behaved as on the other media, but AA failed to produce gas. In other words, it was shown that Cultures a and b were prevented from producing nitrite on the synthetic medium by the

TABLE IV. NITRATE REDUCTION BY *Ps. fluorescens* IN 0.1 PER CT. NITRATE AGAR. FIRST TEST.*

Cultures inoculated in triplicate, but only one tube tested each day. Strength of reaction indicated as follows: +++ very strong, ++ strong, + distinct, T trace, — absent, ? doubtful.

	Medium P.		Medium DM.		Medium DL.			Medium S.						Medium DA.		
	Nitrite present in		Nitrite present in		Nitrite present in			1st day.		2d day.		4th day.		Nitrite present in		
	1 day.	4 days.	4 days.	7 days.	1 day.	2 days.	3 days	Nitrite	Ammonia.	Nitrite.	Ammonia.	Nitrite.	Ammonia.	1 day.	2 days.	3 days.
Culture A	+++	T	—	....	+++	+++	+++	++	?	+++	—	+++	T	++	++	++
Culture B	+++	+++	—	+	+	+++	+++	++	?	++	—	+++	T	++	++	++
Culture a	—	—	T	....	+	+	+	+	?	+	+	+	+	—	—	—
Culture b	—	—	T	....	+	T	T	+	?	+	+	+	+	—	—	—
Culture AA	+	T†	+†	....	++	++	+++†	+++	?	+++	+	+†	++†	....	....	++
Culture BB	++†	T†	—†	....	+	++†	+†	+	++	+	—	+	T	....	†	+†

* In this test the five media were inoculated separately, on different days.
† Gas production shown by presence of cracks in the agar.

addition of ammonium chloride. This test was considered to show that some strains of fluorescent organisms, altho capable of reducing nitrate, do not attack it if there is a more available source of nitrogen present.[14] In this connection it is interesting to note that the nitrite reaction of these two organisms was stronger on the sucrose medium than on the media containing those sugars which had ammoniacal impurities.

Another test (Table V) was run a few months later. This test was a repetition of Tests Nos. 2 to 5, using the same four media together with one other dextrose agar only slightly different (Medium D, Table III). The same six cultures were tested together with four others (laboratory cultures isolated from soil nearly six years previously): two gas-formers CC and DD and two that were nitrite-negative in peptone media, c and d. Of these four cultures, DD was especially interesting. It had been kept under cultivation in the laboratory for the longest time of all, and when first tested on Medium P (nitrate-peptone agar) six years after isolation, was found to be a weak gas-producer. During the next few months its power of gas-production apparently diminished until when the work recorded in Table V was done it failed to produce gas on any medium. The possibility of a contamination being present and causing this discrepancy is not entirely excluded.

The four cultures AA, BB, A, B, a, and b, gave results agreeing fairly well with the earlier tests, except in regard to the gas-production by AA and BB on certain media, a point which seems open to considerable variation. The two old cultures, c and d, were found to be even less vigorous nitrate-reducers than a and b. Culture c produced no nitrite on any medium and only a trace of ammonia on Medium S; Culture d showed nitrite and ammonia on Medium S, but no nitrite on any other medium. Culture c grew very poorly on Medium S, a fact which undoubtedly explains its failure to reduce the nitrate on this synthetic medium.

It was therefore concluded that vigorous growth of the fluorescent organisms is as necessary as it is with the colon organisms, in order to get a satisfactory test for nitrate-reduction; but that a more common cause of error is that cultures do not always reduce the nitrate if a more readily available source of nitrogen is present. In the present work the difference between those cultures that could reduce nitrate under all conditions and those that could do so only in the absence of other sources of nitrogen remained constant; but, as the two sets of cultures resembled each other in all

[14] Another possible explanation is that growth is so rapid in the presence of ammonium chloride that these organisms are able to use up the nitrite as fast as produced. This theory is not a satisfactory explanation, however, because the growth of Cultures a and b was better in the absence than in the presence of ammonium chloride in this medium.

TABLE V. NITRATE REDUCTION BY *Ps. fluorescens* IN 0.1 PER CT. NITRATE AGAR. SECOND TEST.*

Cultures inoculated in triplicate, but only one tube tested each day. Strength of reaction indicated as follows: +++ very strong, ++ strong, + distinct, T trace, — absent, ? doubtful.

Culture.	Medium DM.				Medium D.				Medium DL.				Medium S.								Medium DA.			
	Nitrite present in				Nitrite present in				Nitrite present in				2 days.		4 days.		5 days.		7 days.		Nitrite present in			
	2 days	4 days.	5 days.	7 days.	2 days.	4 days.	5 days.	7 days.	2 days.	4 days.	5 days.	7 days.	Nitrite	Ammonia	Nitrite	Ammonia	Nitrite	Ammonia	Nitrite	Ammonia	2 days.	4 days	5 days.	7 days.
A	+++	++	+++	....	+++	+++	+++	....	+++	+++	++	++	+++	T	++	?	++	T	....	....	+++	+++	+	....
B	+++	+++	+++	....	+++	+	+++	....	+++	+	++	....	+++	T	++	?	++	T	....	....	++	+	+	....
a	+	T	....	+	+	+	....	T	+	T	....	+	++	+	++	?	....	....	++	?	—	—	....	—
b	+	T	....	+	+	+	....	T	+	T	....	T	++	+	++	?	....	....	++	?	—	—	....	—
c	—	....	—	—	—	....	—	—	—	....	—	—	—	T	....	....	—	T	—	—	—	....	—	—
d	—	....	—	—	—	....	—	—	—	....	—	—	+	—	....	....	+	+	T	T	—	....	—	—
AA	++	—†	—†	....	++	++	+++†	....	++	++	++†	....	+++	+	T†	+	—†	+	....	....	++	++	++	....
BB	+++†	+†	....	T†	+++	+†	T†	....	+++	+++†	—†	....	+	T	+	T	+	T	....	....	+++†	+++†	+++†	....
CC	+++	+†	T†	....	+++	+++†	+†	....	+++	+++†	+++†	....	+	T	+	T	+	+	....	....	++	+++†	+++†	....
DD	+	T	....	+	+	—	....	—	+	T	....	—	+	—	+	—	....	....	+	—	—	—	—	....

* In this test the five media were inoculated simultaneously.
† Gas production shown by presence of cracks in the agar.

other respects and as various gradations were found between those that showed no nitrite in the presence of mere traces of ammonia and those that produced it even in the presence of peptone, it does not seem to be a question of two distinct species.

WORK WITH AN ORANGE CHROMOGENIC PSEUDOMONAS.

Another organism chosen for study because of its irregularity in respect to the nitrite test was an orange chromogenic pseudomonas, a form very common in soil and water. It has recently been studied in this laboratory and a description of it has been published.[15] It is concluded to be identical with *Bacillus caudatus* Wright (1895), altho it has a polar flagellum. This organism grows so poorly in liquid media of all sorts that nitrate broth was realized from the beginning to be an unfair medium to use in the nitrate-reduction test, and the early irregular results were considered to be due to the poor growth in this broth. For that reason beef-extract-peptone agar containing 0.1 per ct. nitrate (Medium P, Table 3) was used in subsequent work.

A special study was first made of eight different strains of this organism, four of which strains had been separated into two or three substrains immediately after isolation from the soil, all of these substrains having been kept distinct during the laboratory cultivation. Including these separate substrains, 15 different cultures were studied. It was found (see columns 2–4, Table VI) that they could be divided into two groups, those producing abundant nitrite on nitrate-peptone agar and those showing no nitrite on this medium. The cultures may be listed as follows:

Group 1 — producing nitrite.

A. A typical chromogenic strain.
B. A typical chromogenic strain.
$\left.\begin{matrix}C_1\\C_2\end{matrix}\right\}$ Two substrains of a typical chromogenic strain.
$\left.\begin{matrix}D_1\\D_2\\D_3\end{matrix}\right\}$ Three substrains of a typical chromogenic strain.
$\left.\begin{matrix}X_1\\X_2\\X_3\end{matrix}\right\}$ Three substrains of a non-chromogenic strain.

[15] Bright, J. W. What soil organisms take part in the ammonification of manure? *Jour. Agr. Res.*, 16:313–332, 1919. N. Y. Agr. Exp. Sta., Tech. Bul. 67:5–28. 1919.
This organism was originally named *Bacillus caudatus* by Wright, a name changed to *Pseudomonas caudatus* in the following paper:
Conn, H. J. Taxonomic study of two important soil ammonifiers. *Jour. Agr. Res.*, 16:333–350. 1919. N. Y. Agr. Exp. Sta., Tech. Bul. 67:29–45. 1919.

Group 2 — not producing nitrite.

$\left.\begin{matrix} a_1 \\ a_2 \\ a_3 \end{matrix}\right\}$ Three substrains of a chromogenic strain.

b. A chromogenic strain.

c. A chromogenic strain.

On nitrate-peptone agar the agreement between the different substrains of any one original strain was complete. Some of the strains grew more vigorously than others. In general there was no correlation between vigor of growth and production of nitrite, altho it was striking that after a few months' cultivation in the laboratory all the nitrite-negative cultures died.

These fifteen cultures were tested upon synthetic nitrate media (Columns 5 to 20, Table VI), the same media as used for the fluorescent organisms. Medium DM gave rather surprising results, as nitrite was absent in all but two cases, and in one of these two cases was present in mere traces. This was not due to poor growth in all cases, for very good growth was obtained with Cultures A and B and with all substrains of C and D; whereas Culture X_2 grew poorly but gave the nitrite test. A later repetition of this test (see Table VII) with A, B and all substrains of X showed nitrite to be produced by Cultures A and B, but that the nitrite reaction with Culture B disappeared after the first day. This suggests that the meaning of the negative reaction in the first test (Table VI) may have been in many cases that the cultures were examined after the nitrite had disappeared.

The poor growth of Strains X, a, b, and c, noticed on this medium, was observed on all the other synthetic agars used. This was undoubtedly due to the fact that these media contained no added organic matter except the sugars; for these four strains were found to be unable to attack any sugar.

The cultures were then tested on Medium DL (Columns 7 to 10, Table VI). A distinct nitrite reaction was obtained promptly with Cultures A, B, C_1, C_2, D_1, D_2, and D_3, and on the 7th day with the non-chromogenic strain X_2. No trace of nitrite was observed with any of the cultures which had not produced nitrite in the earlier tests. The correlation between vigor of growth and strength of the nitrite reaction was quite marked. The next test was made upon Medium S, containing sucrose instead of dextrose and lactose, sucrose being used because of its freedom from ammoniacal impurities. Tests for both nitrite and ammonia were made. The results were very striking. Nitrite (at least in traces) was observed with every culture except one of the substrains (X_1) of the non-chromogenic strain. Appreciable amounts of ammonia were observed with every culture, showing nitrate-reduction to have occurred in all

TABLE VI. NITRATE REDUCTION BY THE ORANGE CHROMOGENIC PSEUDOMONADS IN 0.1 PER CT. NITRATE AGAR. FIRST TEST.*

Cultures inoculated in triplicate, but only one tube tested each day. Strength of reaction indicated as follows: +++ very strong, ++ strong, + distinct, T trace, — absent, ? doubtful.

	MEDIUM P.			MEDIUM DM		MEDIUM DL.				MEDIUM S.								MEDIUM DA.	
	NITRITE PRESENT IN			NITRITE PRESENT IN		NITRITE PRESENT IN				1ST DAY.		2ND DAY.		4TH DAY.		7TH DAY.		NITRITE PRESENT IN	
	1 day.	4 days.	7 days.	4 days.	7 days.	1 day.	2 days.	3 days.	7 days.	Ni-trite.	Ammo-nia.	Ni-trite.	Ammo-nia.	Ni-trite.	Ammo-nia.	Ni-trite	Ammo-nia.	4 days.	7 days
Culture A....	++	+++	......	—	......	+	+	++	......	+	+	++	++	++	++	......	......	+	......
Culture B....	T	++	++	—	......	+	+	++	......	+	+	+	++	+	++	......	......	+	......
Culture C_1....	++	+++	+	—	......	+	+	+	......	+	+	++	++	++	++	......	......	+	......
Culture C_2....	+	+++	++	—	......	+	+	++	......	+	+	+	++	+	++	......	......	+	......
Culture D_1....	+	+++	+	T	......	T	+	+	......	+	+	+	++	+	++	......	......	+	......
Culture D_2....	+	++	+	—	......	T	T	+	......	+	+	+	++	+	++	......	......	+	......
Culture D_3....	+	++	++	—	......	+	T	+	......	+	+	+	++	+	++	......	......	+	......
Culture X_1....	—	+++	+++	......	—	—	—	—	—	......	......	—	+	—	+	—	++	......	......
Culture X_2....	—	+++	+++	......	+	—	—	—	+	......	......	T	+	T	+	+	++	—	—
Chlture X_3....	—	+++	+++	......	—	—	—	—	—	......	......	—	+	T	+	—	++	......	......
Culture a_1....	—	—	T	......	—	......	—	—	—	......	......	—	T	—	T	T	+	......	......
Culture a_2....	—	—	T	......	—	......	—	......	—	......	......	—	T	—	+	T	+	......	......
Culture a_3....	—	T	T	......	—	......	—	—	—	......	......	—	T	—	++	T	+	......	......
Culture b....	—	—	T	......	—	......	—	—	—	......	......	++	T	+++	++	......	......	T	+
Culture c....	—	—	T	......	—	......	—	—	—	......	......	—	T	—	+	T	+	......	......

* In this test the five media were inoculated separately, on different days.

cases. As in the previous test, vigor of growth was distinctly correlated with the strength of the nitrite reaction, growth in this case tending to be rather better than on the lactose-dextrose medium.

Later (last two columns, Table VI) a few of the cultures were tested upon the agar (Medium DA) containing ammonium chloride as well as nitrate and dextrose. Nitrite was found with all seven of the strains that showed nitrate on nitrate-peptone agar (Medium P), also with Culture b, the one strain tested that had not shown nitrite on Medium P, but not with the one substrain (X_2) of the non-chromogenic organism that was tested.

A few months later those cultures that were still alive were tested again (Table VII), together with a few other strains of the same organism. This is a very difficult organism to keep living under laboratory conditions, and only cultures A, B, X_1, X_2 and X_3 were still alive. The strains which had failed to produce nitrite on the Medium P had all died. The new cultures studied were:

E, F, G } Three recent isolations from soil, all giving a strong nitrite reaction on Medium P when first isolated.

X_4 A fourth substrain of the non-chromogenic strain, obtained by inoculating X_3 into sterile soil and reisolating after a few weeks.

These nine cultures were inoculated into the same media used in the earlier test and also into Medium D.

The only essential difference noted from the results of the earlier tests was that the non-chromogenic cultures had now lost their power of producing nitrite upon any of the media tested, even including Medium P. Cultures A, X_1, X_2 and X_4 still gave distinct ammonia reactions in Medium S, and X_3 showed a trace of ammonia.

The conclusion drawn from these tests is that this pseudomonad is neither like the colon organisms nor like the fluorescent bacteria in respect to the nitrate-reduction test. Some available organic matter must be present in order to allow good growth; and yet it is possible for some strains to produce good growth without giving the nitrite test. There is evidence that in some cases, at least, this is because the nitrite is converted into ammonia as fast as produced, so that there is no accumulation of nitrite.

CONCLUSION.

As a result of this work the conclusion was drawn that the nitrate-reduction test, as made by testing for nitrite in " standard " nitrate broth after a definite period of incubation, is not as simple as generally supposed. This test is open to several sources of error:

(1) *Poor growth.* Any organism must be tested in some medium in which it makes good growth. If it grows poorly, the results are

TABLE VII. NITRATE REDUCTION BY THE ORANGE CHROMOGENIC PSEUDOMONADS IN 0.1 PER CT. NITRATE AGAR. SECOND TEST.*

Cultures inoculated in triplicate, but only one tube tested each day. Strength of reaction indicated as follows: +++ very strong, ++ strong, + distinct, T trace, — absent, ? doubtful.

	MEDIUM P				MEDIUM DM.				MEDIUM D.			
	NITRITE PRESENT IN				NITRITE PRESENT IN				NITRITE PRESENT IN			
	1 day.	2 days	5 days.	7 days.	1 day.	2 days.	5 days	7 days.	1 day.	2 days.	5 days.	7 days.
Culture A	+++	....	+++	....	+	+	++	....	+	—	+	....
Culture B	T	....	+++	....	+	—	—	....	++	T	++	....
Culture E	+++	+++	+++	....	+++	+++	+	....	+	T	+	....
Culture F	+++	+++	+++	....	+++	+++	++	....	++	+	++	....
Culture G	—	....	....	+++	+	....	—	—	T	....	T	+
Culture λ₁	—	....	....	—	—	....	—	—	—	....	—	—
Culture X₂	—	....	....	—	—	....	—	—	—	....	....	—
Culture λ₃	—	....	....	—	—	....	—	—	—	....	—	—
Culture X₄	—	....	....	—	—	....	—	—	—	....	—	—

TABLE VII.—(*Continued*). NITRATE REDUCTION BY *Ps. caudatus* IN 0.1 PER CT. NITRATE AGAR. SECOND TEST.*

	MEDIUM DL.				MEDIUM S.								MEDIUM DA.			
	NITRITE PRESENT IN				1ST DAY.		2ND DAY.		5TH DAY.		7TH DAY.		NITRITE PRESENT IN			
	1 day.	2 days.	5 days.	7 days.	Nitrite.	Ammonia.	Nitrite	Ammonia.	Nitrite.	Ammonia.	Nitrite.	Ammonia.	1 day.	2 days	5 days.	7 days.
Culture A	++	+	+	....	+	....	+	+	+	+	....	....	+	++	+	....
Culture B	T	+	T	....	++	....	+	+	+	++	....	....	—	....	+	....
Culture E	++	++	+	....	++	....	++	T	+	+	....	....	++	+	+	....
Culture F	T	....	?	....	+	....	++	+	+	+	....	....	++	++	++	....
Culture G	T	....	—	+	+	....	....	....	—	++	+	++	++	....	+	+
Culture X₁	—	....	—	—	—	....	....	....	—	—	—	+	—	....	—	....
Culture X₂	—	....	—	—	—	....	....	....	—	T	—	++	—	....	—	—
Culture X₃	—	....	—	—	—	....	....	....	—	—	—	+	—	....	—	—
Culture X₄	—	....	....	—	—	....	....	....	—	?	—	+	—	....	—	—

* In this test the six media were inoculated simultaneously.

RELATION BETWEEN LACTIC ACID PRODUCTION AND BACTERIAL GROWTH IN THE SOURING OF MILK.*

J. C. BAKER, J. D. BREW AND H. J. CONN.

SUMMARY.

1. Two experiments have been performed in which bacteriological analyses and acid determinations have been made each hour or half-hour during the souring of milk by a pure culture of the lactic acid organism. Six other similar, but less extensive, experiments have been performed to confirm some of the results of the first two experiments.

2. The average size of the group of lactic acid organisms in milk was 1.8 individuals.

3. Microscopic counts have, in general, been about 1.8 times as large as counts made by the plate method. The latter counts have been nearly the same as the counts of " groups " obtained by counting, under the microscope, each isolated organism as well as each group of two or more as a " group." As the microscopic counts of individual bacteria seem to be the most nearly correct, they were used as the basis of the computations.

4. In one experiment, the bacteria were found to multiply very nearly according to geometrical progression, as would naturally be expected. In another experiment, their multiplication lagged so much behind this rate as to show arithmetrical, instead of geometrical, increase. The culture used in the latter experiment produced acid so slowly that the milk was not curdled in 24 hours.

5. As already pointed out by Rahn, there is considerable variation in the rate of acid production per individual cell per hour in the case of different cultures of this organism. This rate proved much lower in case of the organism which multiplied arithmetically than in the case of those multiplying normally. This rate tends to decrease as the curdling point approaches, and, for a vigorous culture, seems to lie between 5×10^{-10} and 10×10^{-10} mgs. lactic acid per hour.

6. The exactness of the formulae for determining fermentative capacity, however, is more apparent than real. The inexactness is due partly to inaccuracies in the bacterial counts and partly to the fact that the rate of multiplication is seldom regular and

* Reprint of Technical Bulletin No. 74, December, 1919.

often not even roughly geometrical, while the formulae apply only to regular geometric increase. To obtain figures of any significance, it is necessary to make hourly or even half-hourly determinations and to obtain in each case the fermentative capacity for a brief period preceding. These individual determinations can then be averaged.

7. In the cases investigated, the ratio of the total acid produced to the number of organisms present proved fairly constant. This ratio is directly proportional (assuming the rate of multiplication to be geometrical) to the amount of acid produced per generation by each individual cell. This ratio was observed to increase as the curdling point approached, and, altho the rate per generation showed considerable fluctuation thruout the course of a single experiment, all the cultures tested gave almost the same ratio when the milk was distinctly sour but not yet curdled.

8. There is a tendency for the acid present, whether measured as titrable acidity or as hydrogen-ion concentration, to increase geometrically until coagulation. To a certain extent, this was true even in the experiment where the bacteria were increasing arithmetically, altho in this case there was considerable lag behind the true curve of geometrical progression.

9. At the curdling point, a cessation in the multiplication of bacteria seems to occur and is accompanied by a noticeable decrease in the rate of acid production. To judge by comparison with some results of Heineman's, this decrease is less noticeable at lower temperatures.

INTRODUCTION.

The rate of growth and production of acid by lactic acid organisms has been the subject of numerous investigations; but none of the previous investigators have used direct methods of counting bacteria, nor have they counted the number of individual cells. This is due to their use of the plate method for counting bacteria, which merely allows the counting of colonies developing on culture plates, some of which colonies may have come from single organisms, others from clumps of several organisms. Rahn,[1] for instance, made a determination of the "fermentative capacity of the individual cell," basing his computations wholly upon the plate count; but his figures are incorrect unless his assumption be true that each colony on the plates comes from a single organism.

Work at this Station, however, shows that this assumption is incorrect. By use of the microscopic technic described by Breed and Brew[2] it is possible to count directly the individual bacteria in

[1] Rahn, Otto. The fermenting capacity of the average single cell of Bacterium lactis acidi. Mich. Agr. Exp. Sta., Tech. Bul. 9, 443–480. 1912.

[2] Breed, R. S., and Brew, J. D. Counting bacteria by means of the microscope. N. Y. Agr. Exp. Sta., Tech. Bul. 49, 1–29. 1916.

milk with considerable accuracy. As shown by Brew and Dotterrer[3], the microscopic count is almost universally higher than the plate count, and the ratio between the two is very inconstant. This is undoubtedly due to the fact that the groups or clumps of bacteria occurring in milk generally produce but a single colony each on the plates. This is borne out by the fact that, if groups and clumps seen under the microscope be counted as single individuals, the count is similar to the plate count but generally somewhat lower. It will, therefore, be seen that Rahn's calculations are incorrect, as are others which assume that the plate count shows the number of bacteria present.

Because of the experience of one of the writers with the microscopic method, it seemed advisable to make a further study of the relation between the number of organisms in milk and the amount of lactic acid formed. It was planned to make hourly and, at times, half-hourly analyses during the eight or ten hours preceding curdling, obtaining data as to the microscopic count, plate count, titrable acidity and hydrogen-ion concentration.

Unfortunately, the work had to be discontinued as soon as it was well started. Everyone who had been taking part in the experimental work left the Station; and, because of the difficult technic required and the amount of labor necessary to control all the conditions, no one was found to carry on the investigation. Inasmuch as the preliminary results obtained are interesting, it was decided to publish the data even tho incomplete; but in the absence of any of the investigators, it was necessary for the work to be written up by one (C) who had not assisted in the actual investigation.

Acknowledgments are due to Dr. R. S. Breed for suggesting the work and for advice in carrying it on; to Miss Jessie Smith for furnishing some of the cultures and obtaining the bacteriological data included in Table VI; and to Prof. J. M. Stetson of Yale University for assistance in connection with the mathematical formulae.

TECHNIC.

The milk used in these experiments was perfectly fresh, coming from a Jersey cow known to be free from udder trouble and giving milk regularly of low bacterial content. It was run thru a carefully sterilized separator to remove the cream, pasteurized by the holding process at 62° C. for one hour, cooled to 25° C., and inoculated with a pure culture of an active lactic acid organism.

The cultures used were isolated and identified as typical lactic organisms by Mr. G. L. A. Ruehle and Miss Jessie Smith of the Station staff. They were selected from a large number of cultures

[3] Brew, J. D., and Dotterrer, W. D. The number of bacteria in milk. N. Y. Agr. Exp. Sta., Bul. 439, 477–522. 1917.

because of the typical fermentation which they produced in milk. The material for inoculation was obtained by growing the organism in pure culture in sterilized milk for long enough to allow much multiplication, but not long enough for curdling to occur. The number of individuals per cubic centimeter in this milk culture was determined by direct microscopic count, and then the flask of pasteurized milk was inoculated with an aliquot of the culture sufficiently large to contain enough bacteria to produce curdling in about 24 hours at 25° C. This inoculation was made at 5:30 P. M., and analyses were begun at 8 o'clock the next morning, taking samples at regular intervals with a sterile pipette.

The microscopic bacterial analyses were made by the method described by Breed and Brew.[4] The plate counts were made by the use of lactose agar with six days' incubation at 21° C. Titration was made with 0.1 N alkali, and results expressed in cubic centimeters required to neutralize 100 c. c. of the milk minus the amount required to neutralize 100 c. c. of the fresh milk. Hydrogen-ion concentration was determined by means of the hydrogen electrode as described by Van Slyke and Baker.[5]

THEORETICAL.

If bacterial growth were perfectly regular, the numbers of bacteria should increase geometrically with a ratio of 2 for each generation. Plotted as a curve, this would be of the logarithmic form with the equation: $\frac{\log B - \log b}{t} = k$, in which k is a constant and is equal to the log. of the ratio of increase per hour.[6] In case there were but one generation per hour, the ratio of increase per hour would be 2, and the value of k would be $\log 2 = .301$. In the souring of milk, however, there are variable factors which may affect the rate of growth, such as decrease in sugar and increase in hydrogen-ion concentration. Decrease in sugar would tend to lessen the rate of

[4] See footnote 2.

[5] Van Slyke, L. L., and Baker, J. C. Free Lactic Acid in Sour Milk. N. Y. Agr. Exp. Sta., Tech. Bul. 65, 21-54, 1918. See p. 30.

[6] The following notation is used both in this equation and thruout the paper:

a = amount of acid present at beginning of period of observation.

A = amount of acid present at end of period of observation.

$S = A - a$ = amount of acid produced during period of observation.

b = number of bacteria present at beginning of period of observation.

B = number of bacteria present at end of period of observation.

t = duration in hours of period of observation.

r = length in hours of the average generation, i. e. the average time required by a cell to complete its life history and become two new cells.

m = amount of acid produced by the average individual per hour.

To conform with this notation it has been necessary to change slightly the formulae taken from other writers.

growth, but, in ordinary souring, this change is so small as to be almost negligible. Change in reaction might cause an increase or a decrease in the rate according to the optimum hydrogen-ion concentration of the organism in question, and in fact might cause first an increase and then a decrease. This factor changes greatly, the hydrogen-ion concentration of milk increasing 100 to 150 times during the souring. Another factor affecting the rate of growth might be the need of organisms to become adapted to the medium of growth after inoculation, and still another factor might be the constantly increasing amount of lactate present in the milk. Any of these factors would operate to prevent the growth curve from being of logarithmic form, and the ratio $\frac{\log B - \log b}{t}$ would not equal a constant thruout the duration of the experiment but would be a decreasing or an increasing quantity according to whether the rate of growth were decreasing or increasing. It would be greater than .301 if there were more than one generation per hour, less than .301 if there were less than one generation per hour.

If the bacteria were increasing regularly, and each cell were producing a constant amount of acid per hour, the total amount of acid would also increase according to geometric proportion. The same equation would hold, substituting amount of acid for numbers of bacteria. The value $k = .301$ would indicate doubling in the amount of acid produced each hour. The ratio would not equal a constant if the increase in acid were not geometrical; but, provided the amount of acid produced per individual organism per hour were constant, the ratios $\frac{\log B - \log b}{t}$ and $\frac{\log A - \log a}{t}$ would increase or decrease at the same rate.

From similar data, Rahn [7] has computed the average fermentative capacity per hour of the individual cell. The formula given by Rahn is: $\frac{S. \log \frac{B}{b}}{t\ (B-b) \log 2}$. He finds considerable variation in the fermentative capacity of different strains, the average of his determinations being 18×10^{-10}. This figure, as already explained, is incorrect because based upon the plate count instead of upon the actual number of bacteria in the milk. The data obtained in the present work enable us to make this computation on the same basis as made by Rahn, for the sake of comparison, and also upon the more correct basis of the microscopic count.

A further error in Rahn's formula is introduced by the fact that he has used simple algebra to solve a problem not adaptable to algebra. To use algebra in obtaining a solution of the problem, it is

[7] See footnote 1.

necessary to assume that each generation is produced suddenly from the preceding generation, with no further multiplication until the next generation appears equally suddenly, and it is further necessary, as Rahn himself admits, to leave the last generation entirely out of account. For the sake of simplification, Rahn assumed that the last generation has not begun to produce acid at the time the final determination is made, and states that, for a more exact

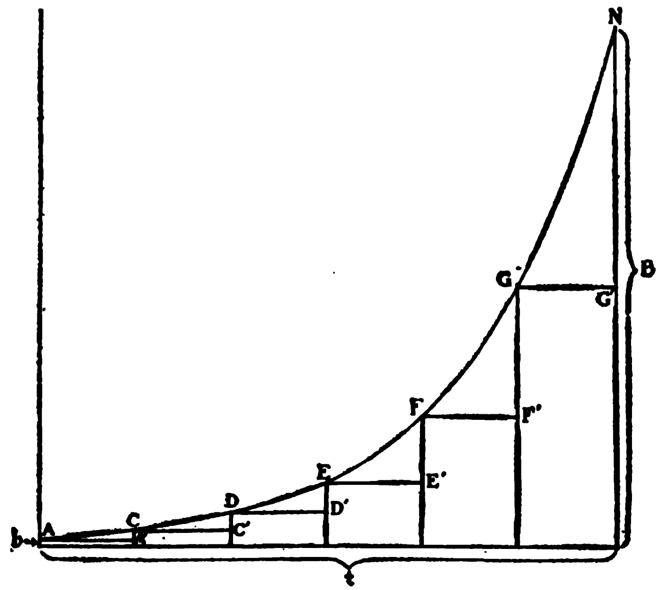

FIG. 1. THEORETICAL CURVE FOR BACTERIAL GROWTH.

estimation, calculus is needed. Buchanan has recently corrected Rahn's figures by the use of calculus, obtaining the formula: $\frac{2.303 \log \frac{B}{b} \cdot S}{t\,(B-b)}$. This formula is only 0.693 times that of Rahn.

The relation between Rahn's and Buchanan's formulae can be best understood if the total number of bacteria acting thruout time t be considered as an area bounded by b, the number of bacteria at the beginning, B, the number at the end, t, the time considered, and the curve AN of bacterial growth (see Fig. 1). Now if all bacteria divide at the same moment at the end of each generation, the curve of growth is a broken line AA′ CC′ DD′ ......N. The area bounded by this broken line can be measured by simple geometry

and algebra, and the quantity thus obtained is exactly that with which Rahn has divided the total acid produced. If, however, bacterial multiplication is constantly taking place so that there is a continual regular increase in their numbers, the curve of growth is the smooth curve ACD........N. The area bounded by this curve requires calculus in order to be measured. It can be seen at a glance that the former area is much smaller than the latter; and, if used as the denominator in the formula for fermentative capacity, the formula will be considerably larger than if the area bounded by the smooth curve be used.

Now, as a matter of fact, we do not know which process of multiplication is the one actually occurring, altho microscopic examination of vigorous cultures suggests that the organisms do not all divide at once and that the smooth curve is the correct one. In this case, Buchanan's formula is the more nearly correct. Neither formula, however, is entirely correct unless the bacteria multiply by geometrical progression, and, as will be shown later on, that is not necessarily the case. Theoretically, indeed, we could expect some or all of the bacteria to become inactive or to grow less vigorously, and hence to cause the curve to lag behind the true curve for geometrical progression. During active fermentation, however, the greatest lag, we could expect would be such as to cause arithmetical progression, in which case the growth curve would be the straight line AN. This is not an extreme case, as shown by Experiment I below (see p. 12). Now the area bounded by this straight line can be determined by plane geometry to be $\frac{t\ (B + b)}{2}$ and the formula in this case for fermentative capacity would be $\frac{2S}{t\ (B + b)}$. This formula gives results lower than Buchanan's, but approaching closer to his the shorter the time considered and the smaller the difference between B and b.

Now, obviously, when we do not know, in a given case, whether the bacteria have actually been increasing geometrically or not, it is impossible to obtain absolutely correct figures by any formula. Exactness is still further out of the question because determinations of bacteria in milk, even when made by the microscope, are but estimates, not actual counts, and exact determinations are impossible without accurate counts of the bacteria. But if all we desire is a rough estimate of fermentative capacity for comparative purposes, one formula gives about as valuable results as the other.

To approximate exactness, it is necessary to make very frequent determinations of bacteria and of acid-production, then to observe whether the rate of multiplication is more nearly geometrical or arithmetical, and to apply the proper formula for each short period,

subsequently averaging the values thus obtained. The present data are well adapted to this form of calculation, as determinations were made each hour or half-hour in Experiments I and II. Upon attempting to make this calculation, however, it was found that the probable error in the bacterial counts was so great in comparison with the difference between successive counts that the results fluctuated enormously. Hence it was found best to figure the fermentative capacity for a period of two hours preceding each determination. The figures thus obtained did not fluctuate greatly, and could be averaged satisfactorily.

Another interesting calculation obtainable from the data secured is the amount of acid produced per individual organism per generation, assuming the rate of multiplication to be geometrical. By the same method of computation used by Buchanan, the equation $m = \frac{S \cdot 2.303 \cdot \log 2}{r\,(B-b)}$ can be obtained.[8]) From this equation we can derive: $mr = \frac{S \cdot 2.303 \cdot \log 2}{B-b}$.
But mr = the amount of acid produced by each organism per generation. Hence the formula $\frac{S \cdot 2.303 \cdot \log 2}{B-b}$ represents the amount produced in a generation by each individual. Provided the initial number of bacteria is unity, or so small as to be disregarded, this value is directly proportional to the ratio $\frac{S}{B}$; if b is a larger quantity, the value in question is proportional to $\frac{S}{B-b} = \frac{A-a}{B-b}$.

EXPERIMENTAL.

The first experiment, made in February, 1917, was carried on with a culture obtained from a butter starter which had been used in the dairy for some time. The results were somewhat unsatisfactory in that the milk had not curdled by 5:30 P. M. on the day after inoculation. At this time the P_H-value had decreased only to 5.12, the curdling point being at about $P_H = 4.7$. The slow rate of fermentation made it impractical to continue this experiment until coagulation occurred. It was concluded that this strain had been cultivated for so long in sour milk as to become attenuated. Accordingly the second experiment, made the following April, was with a freshly isolated culture of the lactic acid organism. This strain curdled the milk between 3:30 and 4 o'clock in the afternoon, that is about 23 hours after inoculation. Subsequent work with these two cultures showed this difference to be constant, one of them

[8] Same notation used in this equation as given in footnote 6.

reaching its final hydrogen-ion concentration some hours earlier than the other. As it has been shown that to maintain the vigor of the lactic acid organism it must always be transferred into fresh milk before the milk becomes very sour, there seems little doubt that continued cultivation of the butter starter in the dairy had attenuated the strain used in the first experiment.[9]

Multiplication of the bacteria.— Table I gives the bacteriological data from the first experiment, and Table II from the second. In the third column of each table is given the microscopic count of individual bacteria, in the fourth the microscopic count of "groups," each isolated organism or group of two or more being counted as a "group." The fifth column gives the plate count. The two following columns give the ratios respectively of the "individual count" and of the plate count to the "group count." The next column gives the value of the ratio $\frac{\log B - \log b}{t}$, which, as already shown, should be a constant, provided the bacteria were increasing in exact geometric ratio.

It will be seen that there is a fairly regular increase in all three of these counts in both experiments, a surprisingly regular increase considering the probable inaccuracies of the counts. There is also a fairly constant ratio between the different counts. The ratio of the "individual count" to the "group count," as explained by Brew and Dotterrer,[10] represents the average size of group present; the ratio of the plate count to the "group count" represents the extent to which the average group broke up during plating. Thus it will be seen that the average size group is about 1.8 in each experiment. In the first experiment there is a tendency for the groups to become larger as the milk sours. The group count and the plate count are nearly the same; in the first experiment the plate count is a little the larger (average ratio 1.14) while in the second experiment it is a little the smaller (average ratio 0.8). This indicates that, provided all the bacteria grew on the plates, the average clump broke up 1.14 times upon dilution in the first experiment and only 0.8 times in the second. The latter ratio (less than unity) would indicate that the bacteria were caused to clump together during the process of plating, a manifestly improbable thing. The probable explanation is that not all living bacteria developed into colonies.

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[9] These same two organisms were used in the recent work by Van Slyke and Baker (reference given in footnote 5). They are referred to on p. 49 of that bulletin, but by a slip, one culture is called *Bacterium lactis acidi* and the other *Streptococcus lacticus*, altho these names are generally regarded as synonyms, and the two cultures, furnished by the bacteriological department, were presumably varieties of the same organism. The one called "*S. lacticus*" is the one used in Experiment I of the present paper which was slower in producing acid.

[10] See footnote 3.

TABLE I.— NUMBERS OF BACTERIA PRESENT DURING THE SOURING OF MILK IN EXPERIMENT I.

TIME OF DAY.	Hours since inoculation.	NUMBER OF BACTERIA PER C. C., AS DETERMINED BY			RATIOS†.			
		MICROSCOPIC METHOD.		PLATE METHOD (P).				
		Individuals (B).	"Groups."* (G).		$\frac{B}{G}$	$\frac{P}{G}$	$\frac{\text{Log B-log b}}{t}$	$\frac{B-b'}{t'}$
5:00 P. M.	0.	6,000	3,000	9,500	....	....	....	....
8:00 A. M.	15.	405,000,000	265,000,000	300,000,000	1.53	1.13	.312	....
9:00	16.	590,000,000	385,000,000	355,000,000	1.57	0.92	.312	185x10[6]
10:00	17.	815,000,000	460,000,000	460,000,000	1.73	1.00	.302	205x10[6]
10:30	17.5	955,000,000	595,000,000	540,000,000	1.60	0.91	.297	220x10[6]
11:00	18.	1,150,000,000	615,000,000	640,000,000	1.88	1.04	.293	250x10[6]
11:30	18.5	1,190,000,000	560,000,000	645,000,000	1.80	0.98	.287	226x10[6]
12:00	19.	1,190,000,000	680,000,000	665,000,000	1.73	0.98	.279	198x10[6]
12:35	19.6	1,280,000,000	655,000,000	1,070,000,000	1.98	1.62	.273	195x10[6]
1:00 P. M.	20.	1,670,000,000	895,000,000	1,220,000,000	1.86	1.37	.272	254x10[6]
2:00	21.	1,690,000,000	830,000,000	1,110,000,000	2.04	1.32	.259	215x10[6]
2:30	21.5	1,800,000,000	1,030,000,000	1,200,000,000	1.75	1.17	.254	215x10[6]
3:00	22.	2,030,000,000	1,010,000,000	1,330,000,000	2.02	1.32	.251	232x10[6]
4:00	23.	2,200,000,000	1,220,000,000	1,640,000,000	1.79	1.33	.242	225x10[6]
5:30	24.5	2,650,000,000	1,180,000,000	1,490,000,000	2.24	1.23	.230	237x10[6]
Average	....	....	....	....	1.82	1.14	....	220x10[6]

* Every isolated individual as well as each group of two or more was counted as a "group."

† In these ratios B = count of individual bacteria. b = initial number of bacteria.
G = "group count." b′ = number of bacteria at the 15th hour.
P = "plate count." t = time considered.
t′ = time since 15th hour.

This difference in ratio may indicate an actual difference between the two cultures used; but it is more probably explained by assuming plating conditions to have favored the growth of the organism more in Experiment I than in Experiment II. Upon noticing this difference, the media records of the laboratory were consulted and it was found that, by an accident, the medium used in Experiment I contained about twice the ordinary amount of lactose (i. e. 2% instead of 1%). As it has been observed before that higher counts can be obtained from milk by increasing the amount of lactose in the culture medium,

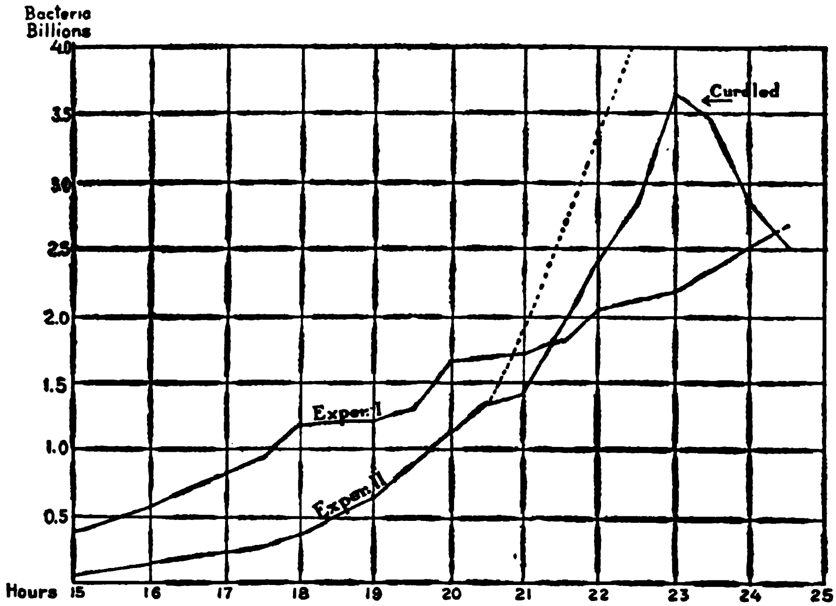

FIG. 2. NUMBERS OF BACTERIA, AS DETERMINED WITH THE MICROSCOPE IN EXPERIMENTS I AND II. THEORETICAL CURVE FOR EXPERIMENT II INDICATED BY DOTTED LINE.

this undoubtedly explains why the plate counts in Experiment I averaged higher in comparison to the "group count" than in Experiment II. The experiments thus indicate that, in the case of the lactic acid organism, the plate count is really a count of the "groups" of bacteria present in the milk, being a little higher or a little lower than the "group count" according to how nearly ideal the plating conditions are for growth of the organisms.

Upon further study of the data, actual differences between the growth of the two cultures is shown. Fig. 2 shows the "individual count" for each experiment plotted out in the form of a curve. At a glance it can be seen that the two cultures have multiplied

according to very different laws. The multiplication in Experiment I is plainly not according to geometric progression, a fact mathematically illustrated by the ratio $\frac{\log B - \log b}{t}$, which, as can be seen from Table I, is constantly decreasing. In fact, the curve for Experiment I is so nearly a straight line that the straight line equation $\left(\frac{B}{t} = k\right)$, which denotes arithmetical progression, can be applied to it. This equation does not apply if the first 15 hours are considered, indicating that, at first, the multiplication was according to some other law. But, by disregarding the first 15 hours, the equation was found to hold very closely. The last column of Table I gives the value of the ratio $\frac{B-b}{t}$, using the value $b = 400{,}000{,}000$ (the number of bacteria in 15 hours) for the initial number of bacteria present. It will be seen that this ratio is very constant, averaging 220×10^6. This means that the bacteria, after the 15th hour, multiplied arithmetically, with an average increase of 220,000,000 per hour. This is quite different from the geometrical progression which theoretical considerations would lead us to expect.

The curve for Experiment II (Fig. 2) is quite different. In fact, this curve very plainly approximates the curve for geometrical progression. From 16 hours until 20.5 hours after inoculation, indeed, a logarithmic curve superimposed upon the curve of growth could not be distinguished from it upon a chart as small as the one used. The equation for the logarithmic curve which fits the given curve so closely is

$$\frac{\log B - \log 57{,}000}{t} = .243 = \log 1.75.$$

In other words, if the initial inoculation had been 57,000 and the numbers had increased 1.75 times each hour, the number present at the 16th hour and from then on until 20.5 hours after inoculation, would have been practically the same as actually found. The initial count, however, was estimated as only 3000, and is fairly accurate because it was calculated by figuring the extent to which a culture of known bacterial content was diluted. Hence it may be concluded that, for the first 16 hours, the hourly ratio was more than 1.75 in order to reach the number of 116,000,000 on the 16th hour. The curve in Fig. 1 shows plainly that after 20.5 hours the rate of growth lagged slightly behind the theoretical rate. To illustrate this point graphically, the curve for the equation just given is continued on the chart in dotted line until the 22d hour, at which time it reaches a point nearly 2 billion higher than the actual count at that hour.

TABLE II.—NUMBERS OF BACTERIA PRESENT DURING THE SOURING OF MILK IN EXPERIMENT II.

TIME OF DAY.	Hours since inoculation.	NUMBER OF BACTERIA PER C. C. AS DETERMINED BY			RATIOS†.			Condition of milk.
		MICROSCOPIC METHOD.		PLATE METHOD (P).				
		Individuals (B).	" Groups "* (G).		$\frac{B}{G}$	$\frac{P}{G}$	$\frac{\text{Log B-log b}}{t}$	
5:00 P. M.	0.	3,000	3,000	....	....	....	....	
8:00 A. M.	15.	35,000,000	21,000,000	32,000,000	1.67	1.53	.275	
9:00	16.	115,000,000	69,000,000	36,000,000	1.68	0.52	.293	Sweet.
10:00	17.	205,000,000	111,000,000	115,000,000	1.83	1.04	.284	
11:00	18.	395,000,000	200,000,000	200,000,000	1.97	0.99	.284	
12:00	19.	645,000,000	260,000,000	215,000,000	2.46	0.82	.280	
1:00 P. M.	20.	1,120,000,000	615,000,000	360,000,000	1.81	0.58	.278	Barely sour.
1:30	20.5	1,330,000,000	740,000,000	395,000,000	1.79	0.53	.275	Distinctly sour.
2:00	21.	1,430,000,000	745,000,000	685,000,000	1.92	0.94	.270	
2:30	21.5	1,950,000,000	1,120,000,000	1,420,000,000	1.75	1.26	.270	Sour, uncurdled.
3:00	22.	2,400,000,000	1,320,000,000	1,130,000,000	1.84	0.86	.269	
3:30	22.5	2,850,000,000	1,585,000,000	1,575,000,000	1.80	0.99	.266	
4:00	23.	3,600,000,000	2,070,000,000	....	1.74	....	.265	Thickening beginning.
4:30	23.5	3,400,000,000	2,090,000,000	3,480,000,000	....	....	....	
5:00	24.	2,850,000,000	1,770,000,000	2,360,000,000	....	....	....	
5:30	24.5	2,500,000,000	1,575,000,000	....	....	....	....	Curdled.
8:30 A. M.	40.	‡4,800,000,000	‡2,550,000,000	2,530,000,000	....	....	....	
9:00	64.	2,550,000,000	1,600,000,000	1,315,000,000	....	....	....	
Average	....	....	....	....	1.86	0.8	.276	

* Every isolated individual as well as each group of two or more was counted as a "group."
† Same notation used as in Table I.
‡ These counts are probably too high; see p. 14.

The curdling point of the milk (at 23 hours) is apparently marked by a very sharp peak followed by a constant decrease in numbers of bacteria during the next hour and a half; but this chart does not include any counts made after the first twenty-four hours. As seen from Table II, the count on the 40th hour was very high (4,800,000,000); but it is recorded in the notes that the distribution of the bacteria was very irregular in this milk on account of the clotting, and more than half of the bacteria counted were in two of the ten fields examined. Leaving these two fields out of account, the individual count would be 3,200,000,000, and the group count 2,000,000,000. Even this count is higher than those made on the 24th and 64th hours, but it does not form an exception to the statement that the highest count occurred at coagulation. No data are yet at hand to show whether this is due to an actual decrease after curdling or to the difficulty in obtaining a count of the entire flora in curdled milk.

Further illustration of the tendency of this culture to multiply geometrically is obtained from the ratio $\frac{\log B - \log b}{t}$, which is fairly constant, as shown by the last column of figures in Table II. These figures were obtained by assuming the initial count to be 3,000 as recorded; but somewhat different results are obtained if the first fairly high count (35,000,000 at the 15th hour) is used,

TABLE III.— DIFFERENT VALUES FOR THE RATIO $\frac{\text{Log B} - \text{Log b}}{t}$ IN EXPERIMENT II, USING DIFFERENT VALUES OF b (I. E. INITIAL COUNT).

TIME OF DAY.	Hours since inoculation	VALUE OF $\frac{\text{Log B} - \text{Log b}}{t}$ WHEN *b* IS		
		3,000	35,000,000 (at 15th hour)	116,000,000 (at 16th hour)
8:00 A. M.	15	.275	....	....
9:00	16	.293	.520	....
10:00	17	.284	.382	.243
11:00	18	.284	.502	.265
12:00	19	.280	.412	.248
1:00 P. M.	20	.278	.509	.246
1:30	20.5	.275	.507	.235
2:00	21	.270	.436	.219
2:30	21.5	.270	.423	.223
3:00	22	.269	.406	.220
3:30	22.5	.266	.388	.214
4:00	23	.265	.377	.214

figuring results from the 15th hour instead of from the beginning. Both sets of figures are listed in Table III, and are rather instructive because they illustrate the difficulty of applying accurate mathematical calculations to bacterial multiplication. It will be seen that those based upon 35,000,000 at the 15th hour are much higher and less constant than those based upon the initial count of 3,000. The cause of this was suspected to be inaccuracy of the 35,000,000 count, which is no higher than the corresponding plate count, and only one third the microscopic count obtained an hour later. Accordingly, another set of figures was prepared based upon the next later count, 116,000,000, figuring from the 16th hour. These figures are given in the last column of the table, and are seen to be fairly constant, almost absolutely constant up to the 21st hour. Altho the counts are evidently too inaccurate to lend themselves perfectly to this kind of computation, the constancy is sufficient to show a very good adherence to the law of geometrical increase.

Another interesting point brought out by Fig. 2 is that, altho at the 15th hour there were many more bacteria in Experiment I than in Experiment II, the curves crossed between the 21st and 22d hour, after which the number in Experiment II was much the greater.

Production of acid.— Table IV gives the acid production in Experiment I, and Table V the acid production in Experiment II, the microscopic counts of individual bacteria being repeated from the previous tables for the sake of comparison. It will be seen that there is a fairly regular increase both in hydrogen-ion concentration and in titrable acidity. Figs. 3 and 4 show that there was a tendency in both experiments for acidity and hydrogen-ion concentration to increase geometrically, but that the rate of increase was much greater in Experiment II than in Experiment I. At the 15th hour there was more acid in Experiment I than in Experiment II, but the greater rate of increase in Experiment II caused the curves to cross between the 20th and 21st hours, after which the amount of acid in Experiment II is much the greater. The acid curves cross an hour before the curves for bacterial numbers (Fig. 2); but, in general, there was a tendency for the acid to be greater at any time in whichever of the two experiments the numbers of bacteria were the higher.

At coagulation in Experiment II there is a sharp break in the curve of titrable acidity an hour later than the peak in the bacterial curve. The break in the C_H-curve is not so marked, but it is plain that the rate of increase is slower after the curdling point. Other experiments have shown the same tendency, this break in the curve always occurring at coagulation.

In the 8th and 9th columns of Tables IV and V are given the fermentative capacity of the individual organism per hour, computed by Buchanan's formula on the basis of the microscopic count. The

TABLE IV.— COMPARISON OF BACTERIAL MULTIPLICATION WITH LACTIC ACID PRODUCTION IN EXPERIMENT I.

TIME OF DAY.	Hours since inoculation.	Microscopic count, individual bacteria per c. c.	Titrable acidity* 0.1 N NaOH per 100 c. c. of milk.	Hydrogen-ion Concentration Expressed as		$\frac{\text{Log A-log a}}{t}$	"Fermentative Capacity," Determined by Formula for				$\frac{A-a}{B-b}$	
							Geometric increase.		Arithmetical increase.			
				P_H.	C_H.		For each 2 hours separately.	From beginning.	For each 2 hours separately.	From beginning.	For each 2 hours separately.	From beginning.
5:00 P. M.	0.	6,000	0.	6.50	$.032 \times 10^{-5}$	....	....	....	....	....	....	....
8:00 A. M.	15.	405,000,000	4.3	6.26	$.055 \times 10^{-5}$	....	....	7.08×10^{-10}	....	1.27×10^{-10}	....	9.5×10^{-10}
9:00......	16.	590,000,000	6.6	6.20	$.063 \times 10^{-5}$	.362	....	7.23×10^{-10}	....	1.26×10^{-10}	....	10.2×10^{-10}
10:00......	17.	815,000,000	8.1	6.08	$.083 \times 10^{-5}$	.290	3.26×10^{-10}	6.22×10^{-10}	2.80×10^{-10}	1.05×10^{-10}	8.33×10^{-10}	9.0×10^{-10}
10:30......	17.5	955,000,000	9.7	6.04	$.091 \times 10^{-5}$	.293	....	6.26×10^{-10}	....	1.05×10^{-10}	....	9.2×10^{-10}
11:00......	18.	1,150,000,000	11.0	6.02	$.096 \times 10^{-5}$	.275	2.36×10^{-10}	5.82×10^{-10}	2.28×10^{-10}	0.96×10^{-10}	7.00×10^{-10}	9.0×10^{-10}
11:30......	18.5	1,190,000,000	13.0	5.97	$.11 \times 10^{-5}$	.268	....	....	....	....	....	9.9×10^{-10}
12:00......	19.	1,190,000,000	14.0	5.88	$.13 \times 10^{-5}$	.246	2.69×10^{-10}	6.80×10^{-10}	2.65×10^{-10}	1.13×10^{-10}	14.3×10^{-10}	10.7×10^{-10}
12:35......	19.6	1,280,000,000	15.9	5.80	$.16 \times 10^{-5}$	.236	2.50×10^{-10}	7.10×10^{-10}	2.49×10^{-10}	1.15×10^{-10}	17.3×10^{-10}	11.2×10^{-10}
1.00......	20.	1,670,000,000	18.0	5.70	$.20 \times 10^{-5}$	.237	2.26×10^{-10}	6.07×10^{-10}	2.24×10^{-10}	0.97×10^{-10}	12.1×10^{-10}	10.8×10^{-10}
2:00......	21.	1,690,000,000	22.0	5.60	$.25 \times 10^{-5}$	.208	2.54×10^{-10}	7.00×10^{-10}	2.50×10^{-10}	1.13×10^{-10}	14.4×10^{-10}	12.1×10^{-10}
2:30......	21.5	1,800,000,000	24.5	5.49	$.33 \times 10^{-5}$	.201	2.54×10^{-10}	7.20×10^{-10}	2.51×10^{-10}	1.14×10^{-10}	14.9×10^{-10}	12.3×10^{-10}
3:00......	22.	2,030,000,000	26.5	5.46	$.35 \times 10^{-5}$	.192	2.08×10^{-10}	6.95×10^{-10}	2.07×10^{-10}	1.07×10^{-10}	21.2×10^{-10}	11.9×10^{-10}
4:00......	23.	2,200,000,000	31.5	5.34	$.46 \times 10^{-5}$	.179	2.20×10^{-10}	7.19×10^{-10}	2.20×10^{-10}	1.12×10^{-10}	16.8×10^{-10}	13.0×10^{-10}
5:30......	24.5	2,650,000,000	41.5	5.12	$.76 \times 10^{-5}$	.165	2.30×10^{-10}	7.19×10^{-10}	2.21×10^{-10}	1.15×10^{-10}	21.8×10^{-10}	14.3×10^{-10}
Average....	....	....	....	......	....	....	2.47×10^{-10}	....	2.40×10^{-10}	....	....	....

* Acidity of sample minus initial acidity.

TABLE V.—COMPARISON OF BACTERIAL MULTIPLICATION WITH LACTIC ACID PRODUCTION IN EXPERIMENT II.

Casein in milk, 3.1 per ct. Initial acidity, 18 c. c. 0.1 N per 100 c. c. milk.

TIME OF DAY	Hours since inocu-lation.	Microscopic count, individual bacteria per c. c.	Titrable acidity* 0.1 N NaOH per 100 c. c. of milk.	HYDROGEN-ION CONCENTRATION EXPRESSED AS		$\frac{\text{Log A-log a}}{t}$	"FERMENTATIVE CAPACITY" DETERMINED BY FORMULA FOR GEOMETRIC INCREASE, ON BASIS OF			$\frac{A-a}{B-b}$	
				PH.	CH.		MICROSCOPIC COUNT. For each 2 hours separately.	MICROSCOPIC COUNT. From beginning.	PLATE COUNT. From beginning.	For each 2 hours separately.	From beginning.
5:00 P. M.	0.	3,000	0.	6.50	$.032 \times 10^{-5}$	....	....	....	....	....	....
8:00 A. M.	15.	35,000,000	1.0	6.43	$.037 \times 10^{-5}$	....	....	16.1×10^{-10}	17.4×10^{-10}	....	25.4×10^{-10}
9:00	16.	115,000,000	2.2	6.39	$.041 \times 10^{-5}$	.305	....	11.4×10^{-10}	....	....	16.9×10^{-10}
10:00	17.	205,000,000	3.5	6.31	$.049 \times 10^{-5}$	.272	11.7×10^{-10}	10.1×10^{-10}	....	14.7×10^{-10}	15.4×10^{-10}
11:00	18.	395,000,000	5.3	6.21	$.062 \times 10^{-5}$	.245	6.15×10^{-10}	7.91×10^{-10}	....	11.1×10^{-10}	12.0×10^{-10}
12:00	19.	645,000,000	9.2	5.91	$.102 \times 10^{-5}$	.243	6.70×10^{-10}	8.30×10^{-10}	....	13.0×10^{-10}	12.6×10^{-10}
1:00 P. M.	20.	1,120,000,000	15.0	5.75	$.108 \times 10^{-5}$	.235	6.78×10^{-10}	7.74×10^{-10}	....	13.4×10^{-10}	11.9×10^{-10}
1:30	20.5	1,330,000,000	18.8	5.58	$.265 \times 10^{-5}$	.232	....	8.05×10^{-10}	....	....	12.6×10^{-10}
2:00	21.	1,430,000,000	23.7	5.46	$.35 \times 10^{-5}$	.229	6.65×10^{-10}	9.30×10^{-10}	....	18.5×10^{-10}	14.7×10^{-10}
2:30	21.5	1,950,000,000	30.2	5.33	$.47 \times 10^{-5}$	.228	....	8.68×10^{-10}	....	....	13.6×10^{-10}
3:00	22.	2,400,000,000	36.2	5.18	$.66 \times 10^{-5}$	.223	5.67×10^{-10}	8.40×10^{-10}	....	16.6×10^{-10}	13.3×10^{-10}
3:30	22.5	2,850,000,000	45.6	4.98	1.05×10^{-5}	.221	6.05×10^{-10}	8.80×10^{-10}	15.2×10^{-10}	17.6×10^{-10}	14.2×10^{-10}
4:00	23.	3,600,000,000	55.8	4.73	1.90×10^{-5}	.218	6.15×10^{-10}	8.48×10^{-10}	....	14.8×10^{-10}	13.7×10^{-10}
4:30	23.5	3,400,000,000	60.2	4.73	1.90×10^{-5}	.210	....	....	....	....	....
5:00	24.	2,850,000,000	64.0	4.57	2.70×10^{-5}	.201	....	....	....	....	....
5:30	24.5	2,500,000,000	64.3	4.56	2.75×10^{-5}	.192	....	....	....	....	....
8:30 A. M.	40.	4,800,000,000	83.5	4.32	4.8×10^{-5}	.0785	....	....	....	....	....
9:00 A. M.	64.	2,550,000,000	97.3	4.17	6.8×10^{-5}	.046	....	....	....	....	....
From 15th to 23d hour			....	....	....	....	....	8.00×10^{-10}	13.5×10^{-10}	....	15.4×10^{-10}
Average			....	....	....	....	6.98×10^{-10}	....	....	15.0×10^{-10}	....

* Acidity of sample minus initial acidity.

figures given in the 8th column are in each case computed for the two hours preceding. At the end of the table is given the average of these figures. In the 9th column the fermentative capacity is figured from the beginning in each case, using 6,000 and 3,000 respectively as the initial counts. There is a noticeable discrepancy between these figures and the average of those in column 8. This

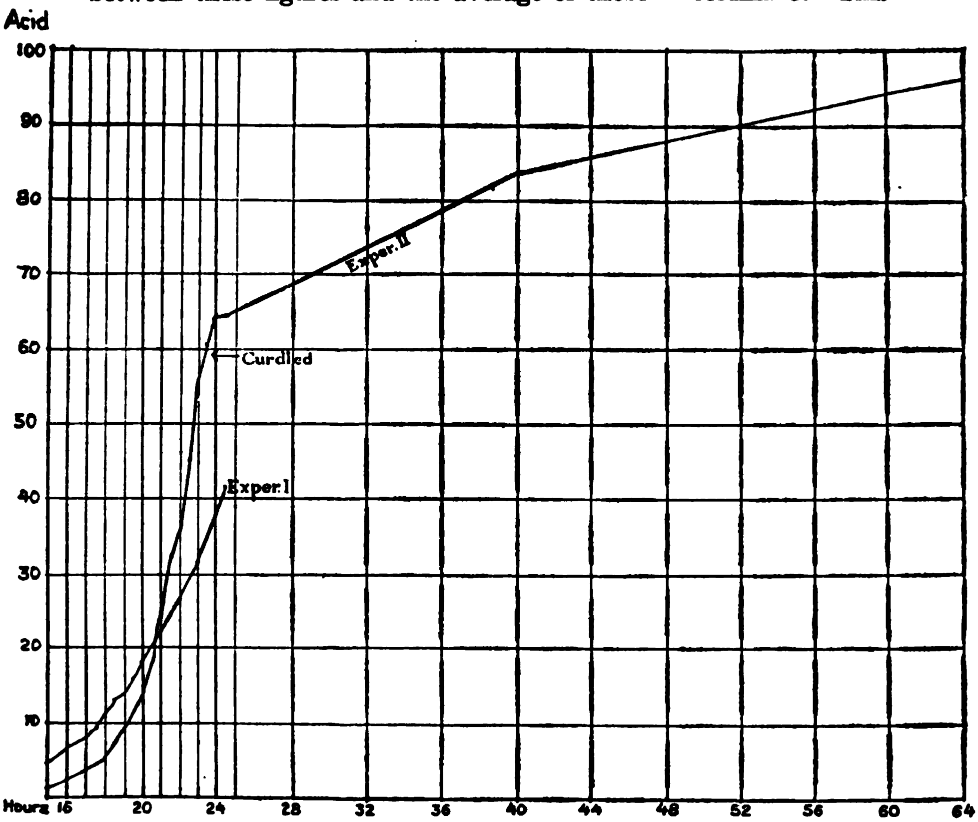

FIG. 3. CUBIC CENTIMETERS OF NORMAL ACID PER LITRE PRODUCED IN EXPERIMENTS I AND II.

discrepancy indicates that Buchanan's formula does not give satisfactory results unless the individual determinations are quite frequent.

In Experiment I this discrepancy is much greater than in Experiment II, because the formula is adapted to geometrical increase only, and the increase was arithmetical in the first experiment. To eliminate this error, the formula for arithmetical increase was also used in the first experiment, and is given in column 10 computed for each

two hours separately, and in column 11 computed from the beginning in each case. It will be seen that the discrepancy between the values in column 11 and the average of those in column 10 is much less than between the similar sets of values when the other formula was used, this apparently indicating that the formula for arithmetical increase gives more nearly the correct figures.

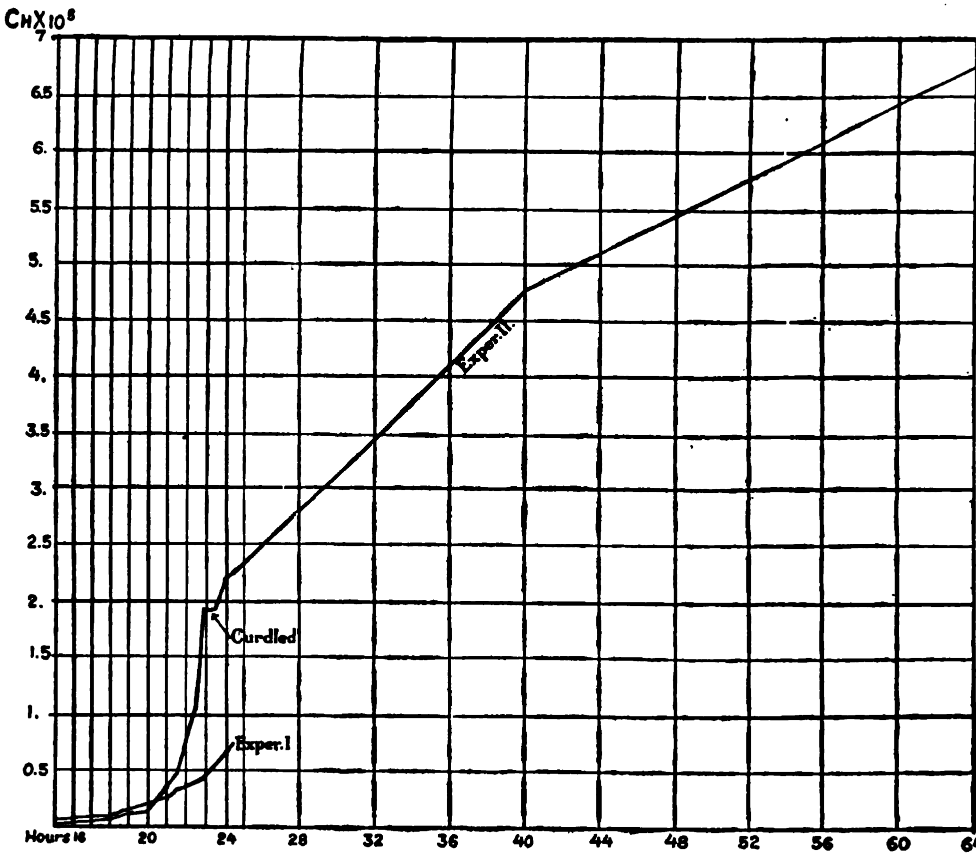

FIG. 4. HYDROGEN-ION CONCENTRATION OF THE MILK IN EXPERIMENTS I AND II.

As we have already seen, none of these values can be considered to have much mathematical accuracy. The nearest we can come to accuracy is to average the value for the first fifteen hours with the average of the values for the following two-hour periods. In Experiment II this can be done simply by averaging 16.1 x 10^{-10} (for the first 15 hours) with 6.98 x 10^{-10} (the average for the next eight hours),

thus obtaining the value 12.9×10^{-10}. In Experiment I there is greater difficulty. Plainly, the arithmetical formula gives best results after the 15th hour, but, as we have already seen, the rate of bacterial multiplication must have been greater before the 15th hour, and perhaps was geometrical during this early period. We have to choose, therefore, between the values 7.08×10^{-10} and 1.27×10^{-10} for the first fifteen hours, according as we decide upon the geometrical or the arithmetical formula, and we have no way of telling which is correct. Under the circumstances, the best that can be done is to take the mean value between these two, namely 4.18×10^{-10}, and average this with the average value for the two hour periods during the next 9.5 hours, namely 2.4×10^{-10}. The value thus obtained for Experiment I is 3.49×10^{-10}, much lower than the corresponding value for Experiment II.

In order to see how these results compare with Rahn's, a column is added to Table V giving the fermentative capacity figured on the basis of the plate count. It seemed worth while to make only three calculations; one for the first 15 hours, the other from the beginning until 22.5 hours after inoculation (the time of the last plate count before coagulation), and the third from the 15th hour until coagulation. The first of these two values is 17.4×10^{-10}, the second 15.2×10^{-10}, and the third 13.5×10^{-10}. Multiplying by 1.443, the ratio of Rahn's formula to Buchanan's, these values become 25.1×10^{-10}, 21.9×10^{-10} and 19.5×10^{-10}, respectively. The last figure is very close to the average (18×10^{-10}) of Rahn's figures. In other words the culture used in this experiment had almost exactly the same fermentative capacity as the average of Rahn's cultures. Rahn's values, however, are about twice too high, partly because he used the wrong formula, and partly because his results were figured on the basis of the plate count instead of a count of individual bacteria. The fermentative capacity of the organism used in Experiment I is obviously much lower, and no effort has been made to compare it with Rahn's figures, altho some of Rahn's strains were equally low in fermentative capacity.

In the last two columns of each table is given the ratio of the acid to the number of bacteria. In the first of these two columns the ratio is figured for each period of two hours, dividing the increase in acid by the increase in bacteria, i. e. $\frac{A-b}{B-b}$. In the second column is given the ratio of total acid to total numbers of bacteria. Now we have already found that, if the bacteria are increasing geometrically, the amount of acid produced per individual cell per generation is $\frac{S \cdot 2.303 \cdot \log 2}{B-b}$. As $2.303 \cdot \log 2$ is a constant (0.696), and $S = A-a$, the ratio given in these columns, is directly proportional to the amount of acid produced per generation by each organism, provided they be

increasing geometrically. This method of increase is the actual one before coagulation in Experiment II, as we have already seen. In Experiment I, where the increase is arithmetical, the length of generation cannot be computed, and is probably inconstant; so, for this experiment, the figures do not have the same significance.

Altho showing considerable fluctuation, there is a tendency for the ratio $\frac{A-a}{B-b}$ to increase up to coagulation (altho this is not true in Experiment II until the 18th hour). It is also striking that, after the milk has become noticeably sour (at about $P_H = 5.4$), the ratio figured from the beginning $\left(\text{i. e. } \frac{A}{B}\right)$ is almost the same in both cases (about 13.5×10^{-10}). This similarity in the final ratios is in considerable contrast to the great difference in the hourly fermentative capacity of the two cultures and seems to indicate that, altho each cell of the first culture produced less acid per hour than each cell of the second culture, the individual cells of both cultures produced about the same amount per generation. Unfortunately, however, on account of the peculiar rate of increase of the first culture, we do not know the significance of the ratio $\frac{A}{B}$ in the first experiment and cannot accept the above statement unreservedly.

A further series of less intensive experiments was run to see whether this ratio is always as constant in milk soured, but not yet curdled, by a pure culture of this organism. The results, given in Table VI, show the data obtained in five experiments, carried on much like the first two, except that hydrogen-ion concentration was not determined, and fewer analyses were made. A different strain of the lactic acid organism (each a typical culture) was used in each experiment. Each of the five experiments in which more than one analysis was made shows an increase in the ratio $\frac{A}{B}$ thruout the period of observation. This ratio is fairly constant, however, and is not far from 13.5×10^{-10}, the ratio found at the end of the first two experiments. The amount of acid produced per cell per hour, figured by Buchanan's formula, shows rather more variation than this, as can be seen from column 6 of the table.

DISCUSSION.

From the above facts it is plain that an active culture of the lactic acid organism in milk does not necessarily multiply regularly so as to increase geometrically. It generally lags behind the theoretical rate of multiplication, and may lag to such an extent as to show arithmetical, instead of geometrical, increase. This lag is probably due to some of the individuals becoming inactive or even

Table VI.—Experiments III to VII. Comparison Between Microscopic Count (of Individual Bacteria) and Acidity of the Milk.

	Hours since inoculation.	Numbers of individual bacteria per c. c. of milk.	Titrable acidity, 0.1 N NaOH per 100 c. c. of milk.	"Fermentative Capacity" Determined by Formula for Geometric Increase.		$\frac{A-a}{B-b}$	
				Intermediate.	From beginning.	Intermediate.	From beginning.
Experiment I	15.5	540,000,000	8.0	....	11.4×10^{-10}	....	13.2
	16.5	1,050,000,000	15.2	8.45×10^{-10}	10.9×10^{-10}	13.2	13.0
	17.5	1,740,000,000	25.6	3.49×10^{-10}	10.8×10^{-10}	13.6	13.2
Experiment II	20.7	1,860,000,000	30.8	....	10.2×10^{-10}	....	14.8
	21.1	2,060,000,000	35.6	5.60×10^{-10}	10.7×10^{-10}	21.6	15.5
Experiment III	19.	860,000,000	11.6	....	8.7×10^{-10}	....	12.3
	21.6	1,850,000,000	28.9	8.00×10^{-10}	9.4×10^{-10}	15.6	13.5
Experiment IV	20.8	1,500,000,000	22.0	....	9.0×10^{-10}	....	13.1
	21.8	1,900,000,000	32.8	5.7×10^{-10}	10.3×10^{-10}	27.0	17.3
	22.5	2,400,000,000	42.0	5.5×10^{-10}	10.3×10^{-10}	18.4	17.5
Experiment V	23.2	1,240,000,000	16.8	....	7.35×10^{-10}	....	12.1
	24.	1,285,000,000	18.5	17.6×10^{-10}	7.6×10^{-10}	34.0	12.8
Experiment VI	23.2	1,700,000,000	20.0	....	6.55×10^{-10}	....	11.8
	24.	2,040,000,000	28.0	6.3×10^{-10}	7.5×10^{-10}	31.0	12.5

dying. It is hard, indeed, to conceive of arithmetical increase except upon some such assumption. The only other possible assumption is that the rate of multiplication is constantly and regularly decreasing. It is interesting to note that the culture multiplying arithmetically is the one which showed the smallest production of acid per cell per hour, and is, therefore, probably the least vigorous of the cultures studied.

Altho, in a given experiment, the amount of lactic acid produced per cell per hour may remain fairly constant, it seemed to vary more with the different cultures investigated than did the amount pro-

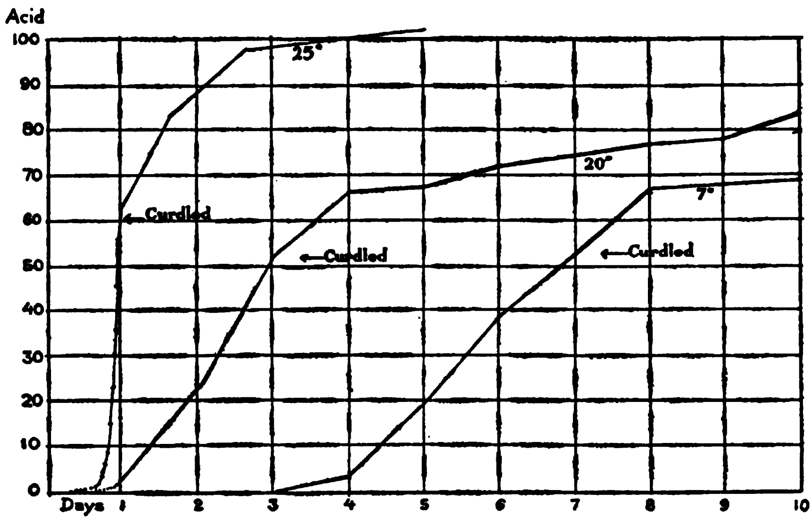

FIG. 5. CUBIC CENTIMETERS OF NORMAL ACID PER LITRE PRODUCED IN EXPERIMENT II AT 25° C. COMPARED WITH HEINEMANN'S SIMILAR EXPERIMENTS AT 20° C. AND AT 7° C.

duced by each cell per generation. There was a tendency for the amount produced per hour (by each cell) to decrease thruout the experiment, while the amount per generation (by each cell) tended to increase.

When coagulation occurs, the number of bacteria present has been found to stop increasing or even to decrease. Coagulation must bind the organisms in place, and this must soon slow the rate of growth due to the formation of colonies, each surrounded (on account of the slackening of diffusive processes in the milk) by a sphere depleted in foodstuff and with an excess of metabolic products. A sharp break in the acid curve was observed to occur about an hour later.

Heinemann [11] has furnished data as to the accumulation of acid by this organism at 20° and 7° C. His figures are given in Table VII, and in Fig. 5 the same data are plotted in the form of curves, compared with Experiment II above, which was carried on at 25° C.

Table VII.— Acidity of Inoculated Raw Milk Incubated at 20° C. and at 7° C According to Heinemann.

Initial acidity 13.1 c. c. 0.1N per 100 c. c. milk.

Days.	Number of c. c. 0.1 N NaOH Required to Neutralize 100 c. c. Milk.	
	20° C.	7° C.
0	0	0
1	3.2	0
2	23.4	0
3	51.9 (curdled)	0
4	65.9	4.4
5	67.1	18.9
6	71.9	39.6
7	73.9	52.4 (curdled)
8	76.9	61.9
9	78.6	67.9
10	83.9	69.1

Apparently the break in the acid curve is not so marked at the lower temperatures, possibly because the slower growth of the organisms requires less foodstuff and its needs are better supplied by the slackened diffusive processes in the curdled milk than when the temperature is higher and the growth more rapid.

[11] P. G. Heinemann. The variability of two strains of Streptococcus lacticus. J. Inf. Dis., 16, 221–289. 1915. See p. 287.

REPORT

OF THE

Department of Botany.

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(See also Report on Inspection Work.)

REPORT OF THE DEPARTMENT OF BOTANY.

MISSING HILLS IN POTATO FIELDS: THEIR EFFECT UPON THE YIELD.

F. C. STEWART.

SUMMARY.

The experiment here described was designed to show how much of the loss due to missing hills is made up by the increased yield of adjoining plants.

At planting time each of 360 potato tubers was divided lengthwise into two equal pieces. The 720 seed-pieces so obtained were planted 15 inches apart in the row in groups of four with blank spaces of 30 inches between groups. In other words, every fifth hill was a missing hill. Each group of four contained the two pairs of seed-pieces from two tubers. Hence, one member of each pair of the resultant plants adjoined a missing hill. The difference between the yield of this member (the exterior plant) and the yield of its mate (the interior plant) was taken as the measure of the influence of the missing hill.

Data were obtained from 351 pairs of plants. In weight of total yield the exterior plants outyielded the interior ones by 23.2 per ct. on the average. Accordingly, the answer given by the experiment is that, under conditions such as obtained in this experiment, the loss from missing hills is offset to a considerable extent by the increased yield of adjoining plants. In the case of a skip containing a single missing hill the two adjoining plants (one on either side) together make up 46.4 per ct. of the loss in total yield and a little more in yield of marketable tubers. Skips of more than one hill are, probably, a total loss except for 46.4 per ct. of the yield of one plant.

The number of tubers per plant was 20.7 per ct. greater for the exterior plants than for the interior ones and the tubers were of larger average size.

As a sort of check on the experiment, an attempt was made to

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determine the magnitude of the variation in yield between the two members of a pair of plants from the same tuber when grown under conditions as nearly parallel as they could be made. For this purpose pairs of seed-pieces similar to those used in the experiment proper were planted in continuous rows without blank spaces. Data were obtained from 85 pairs of plants. In different pairs the difference in total yield varied from 0 to 66.7 per ct. of the mean yield of the two plants of the pair, the average being 20.7 per ct. Such a wide variation in yield between plants under supposedly parallel conditions indicates that there are factors having a very important bearing on the yield of potatoes which are either unknown or not estimated at their proper value.

INTRODUCTION.

Potato growers, generally, appear to take it for granted that the effect of missing hills or "skips" in potato fields is to lower the yield more or less. Accordingly, efforts are made to obtain as nearly a full stand of plants as may be possible. Concerning the amount of the loss, opinions differ widely. Some assume that a missing hill is a total loss. Others hold that a large part of the loss is made up by the increased yield of the adjacent plants. Probably, the amount of the loss varies considerably with the variety, the distance between hills, and the cultural, soil and weather conditions; but there seem to be few experimental data upon which to base an opinion. The data given by Fitch and Bennett[1] are the only ones which have come to the writer's attention.

During the past season the writer conducted an experiment designed to shed additional light upon this subject.

PLAN AND METHODS.

The experiment plat contained 902 plants in twelve rows which were three feet apart and about 112 feet long. The soil of the plat was a moderately fertile clay loam containing a considerable number of small stones. In 1917 it had received an application of stable manure and grew a crop of tomatoes. No manure or fertilizer of any kind was applied in 1918. The plat was plowed in the fall and

[1] Fitch, C. L., and Bennett, E. R. The potato industry of Colorado. Col. Sta. Bul. 175:65–68. 1910.

again in the spring. Planting was done by hand on June 3 in furrows opened with a plow. Owing to recent heavy rains the soil was somewhat lumpy and not as fine as one might wish, yet it was in fairly good condition for planting. The seed tubers were of the variety Sir Walter Raleigh, and selected for their regularity in shape. In weight they varied from two to four ounces. In Rows I to X, inclusive, planting was done in the following manner: Each tuber was cut lengthwise into two parts as nearly equal in weight as they could be made without resorting to the use of scales, and the pieces placed in the furrow fifteen inches apart in groups of four with a space of thirty inches between groups. To avoid error thru mixing of the seed-pieces before planting, the two pieces of one tuber were planted before the next tuber was cut. The seed-pieces were covered by means of a hoe.

In each group the first piece (plant or hill) will be designated as a, the second a', the third b', and the fourth b. The spaces between groups serve the purpose of missing hills. In other words, every fifth hill was a blank or missing hill. The accompanying figure illustrates the arrangement.

X	X	X	X	O	X	X	X	X	O	X	X	X	X	O	X	X	X	X
a	a'	b'	b		a	a'	b'	b		a	a'	b'	b		a	a'	b'	b

FIG. 6.—SHOWING METHOD OF PLANTING IN ROWS I—X.

In each group a and a' were pieces of the same tuber; likewise, b' and b were from the same tuber. Under parallel conditions a and a' should give the same yield; also, the yield of b' should equal that of b. But if missing hills affect the yield of adjoining plants a should outyield a' and b outyield b'.

Of course it is not to be expected that a and b plants would outyield their mates in every case, even tho influenced by adjoining hills, because of the impossibility of making other conditions strictly parallel. However, this difficulty may be overcome by averaging the results from a large number of pairs.

In order to determine how successfully the factors causing inequality were eliminated from the experiment Rows XI and XII were planted in such a way as to form a sort of check. In these rows the seed-pieces were planted fifteen inches apart without any missing hills as shown in Figure 6.

X X X X X X X X X X X X X X X X X X

c c′ c c′ c c′ c c′ c c′ c c′ c c′ c c′ c c′

FIG. 7.—SHOWING METHOD OF PLANTING IN THE CHECK, ROWS XI AND XII.

In Figure 7 the two pieces of each seed-tuber are designated *c* and *c′*. If all conditions have been made parallel, *c* should give the same yield as *c′* in every case.

While the plants were coming up each one was carefully examined to make sure that none were covered by stones or hard lumps of soil. Every one of the 902 seed-pieces produced a plant.

In the whole experiment there were 451 pairs of plants. On July 8, when the plants were 6 to 9 inches high, the members of each pair were compared as to size. Pairs in which the two plants were materially unequal in size were marked for rejection at digging time, because an inequality at this stage of growth must be due to some other cause than the influence of a missing hill. It was too early for plants adjacent to missing hills to have made any use of the blank space. Their roots had not yet extended so far. Fourteen pairs of plants, four of which were in the check, were marked for rejection.

It having been observed that, frequently, one member of a pair of plants had more stalks than its mate — a condition which would tend to produce inequality in the yield — some of the stalks were removed to make the number equal. In most cases equality in this respect was secured by removing one or more stalks from the plant having the larger number; but, sometimes, stalks were removed from both plants. Always, the size of the stalks was considered, the aim being to leave the two plants of a pair with the same number of stalks of similar size. This was done when the plants were 6 to 9 inches high, and before the inequality in number of stalks could have had much effect. In the great majority of cases the stalks pulled up without breaking and without disturbing the seed-piece. The number of stalks most common was four to six.

The plants were cultivated three times with a one-horse cultivator, and hoed thoroly twice. They were sprayed with bordeaux mixture three times — twice, when the plants were small, with a horse-power sprayer, and once later, very thoroly, with a knapsack sprayer. In the third cultivation a few branches were broken. Also, in the second spraying some branches were injured by the wheels of the sprayer. It would have been better if all of the cultivation and spraying had been done by hand.

No appreciable damage was done by insects or blight of any kind. The foliage was nearly perfect up to October 1, but declined rapidly after that date and was all dead by October 15. The tubers were free from rot and scab.

WEATHER CONDITIONS.

The weather conditions were not unusual in any respect. In June, July and September there was sufficient rain for the needs of the plants. During a sudden heavy shower on July 27, the leaves on prostrate branches of some of the plants were partially buried under soil, but as most of them were carefully removed while the soil was yet soft it is believed that only slight injury resulted. August was too dry for the best results with potatoes, but not excessively dry. There were a few days of very hot weather about August 1. Fortunately, the plants escaped the frost of September 10 which killed potatoes over the greater part of the State.

LEAF ROLL.

About the middle of July it became evident that a considerable number of plants in the experiment plat were affected with leaf roll, one of the so-called degeneration diseases. The affected plants were much smaller than normal plants, of lighter green color, the branches and leaf petioles were shortened and the margins of the leaves rolled upward.

On July 15, when the normal plants were 9 to 16 inches high, each plant in the experiment plat was carefully examined, and its condition of health recorded. At this time the appearance of diseased plants contrasted strongly with that of normal plants, making diagnosis quite easy. However, there were found a few plants which it was necessary to record as "doubtful" it being impossible to determine definitely whether they were normal or mildly affected with leaf roll. It was expected that a second examination of the doubtful plants, later in the season, would clear up their status; but this was neglected for a time, and the heavy rain of July 27 so altered the appearance of the foliage that it was impossible to make a dependable diagnosis after that date.

According to the record made on July 15, there were 94 plants (10.4 per ct. of the total) definitely affected with leaf roll. Twelve

of the leaf roll plants were about two-thirds the size of normal plants, while the remaining 82 were recorded as small or very small. Several were worthless dwarfs. Six plants were recorded as "doubtful."

The diseased plants invariably occurred in pairs. When the plant from one-half of a seed-tuber was affected, its mate from the other half of the tuber was also affected and, usually, to the same degree. Of the 47 pairs of affected plants only four were rejected for inequality as described on page 48. However, a fifth pair was rejected at digging time because of an extraordinary difference in yield, one of the plants yielding two and one-half times as much as its mate.

Altho the leaf roll plants lived as long as the normal plants, the majority of them never attained one-half the size of normal plants. The yield was small, and the tubers set close to the stem on very short stolons as is usually the case in leaf roll.

HARVESTING THE CROP AND RECORDING THE DATA.

All operations under this head were performed by the writer, himself. Thoro precautions were taken to avoid errors.

First, a record was made of the product of each plant — the number of tubers and their total weight to the nearest half ounce. Tubers of all sizes above about one-fifth ounce in weight were included. The data so obtained constitute the "hill record." (Table I.)

After being counted and weighed, the tubers of normal *a* and *b* plants were put into one crate; the tubers of leaf roll *a* and *b* plants into another; those of normal *a′* and *b′* plants into a third; and those of leaf roll *a′* and *b′* plants into a fourth crate.

Secondly, each of the above four lots of tubers was sorted into three classes, and the tubers of each class counted and weighed. The first class contained all tubers weighing over two ounces; the second class all tubers weighing between one and two ounces; and the third class all tubers under one ounce in weight. The data so obtained constitute the "bulk record." (Table II.)

Full, detailed records are given only for the experiment proper, a summary being deemed sufficient for the check.

THE HILL RECORD.

Table I shows the hill record of the 351 pairs of plants in the experiment proper. Plants adjoining missing hills are designated " exterior plants " and those not adjoining missing hills " interior plants." Figures in the column headed " difference in yield " show the difference in the yield of the two members of each pair of plants. A minus (—) sign indicates that the difference was in favor of the interior plant. Figures in the column headed " percentage of increase or decrease in yield " show the difference in yield expressed in percentage of the yield of the interior plant. Plants affected with leaf roll are starred.

TABLE I.— HILL RECORD IN MISSING HILL EXPERIMENT: SHOWING THE YIELD OF 351 PAIRS OF POTATO PLANTS.

EXTERIOR PLANTS.			INTERIOR PLANTS.			Difference in yield ($a - a'$ or $b - b'$).	Percentage of increase or decrease in yield ($\frac{a-a'}{a'}$ or $\frac{b-b'}{b'}$).
Plant.	Weight of tubers.	Number of tubers.	Plant.	Weight of tubers.	Number of tubers.		
	Oz.			Oz.		Oz.	Per ct.
a	24.5	6	a'	19.5	6	5.0	25.6
b	24.5	7	b'	14.5	4	10.0	69.0
a	19.0	5	a'	14.5	5	4.5	31.0
b	13.0	7	b'	12.5	4	0.5	4.0
a	18.0	4	a'	19.5	6	—1.5	—7.7
b	19.5	6	b'	16.0	5	3.5	21.9
a	24.5	7	a'	18.0	7	6.5	36.1
b	22.0	6	b'	23.0	8	—1.0	—4.3
a	24.0	7	a'	16.0	5	8.0	50.0
b	26.5	7	b'	16.5	4	10.0	60.6
a	25.0	7	a'	18.0	6	7.0	38.9
b	20.5	7	b'	16.5	5	4.0	24.2
a	21.0	5	a'	20.5	4	0.5	2.4
b	25.0	8	b'	18.0	5	7.0	38.9
a	24.5	5	a'	19.0	6	5.5	28.9
b	20.5	5	b'	18.0	6	2.5	13.9
a	20.5	5	a'	16.0	5	4.5	28.1
b	22.0	4	b'	16.0	6	6.0	37.5
a	25.0	6	a'	13.5	3	11.5	85.2
b	25.0	8	b'	22.5	7	2.5	11.1
a	24.0	5	a'	23.0	7	1.0	4.3
b	27.0	7	b'	16.5	5	10.5	63.6
a	18.0	4	a'	13.0	5	5.0	38.5

TABLE I (*continued*).

Exterior Plants.			Exterior Plants			Difference in yield ($a - a'$ or $b - b'$).	Percentage of increase or decrease in yields ($\frac{a-a'}{a'}$ or $\frac{b-b'}{b'}$).
Plant.	Weight of tubers	Number of tubers	Plant	Weight of tubers	Number of tubers		
	Oz.			*Oz.*		*Oz.*	*Per ct.*
*b	14.5	7	*b'	6.0	4	8.5	141.7
a	22.0	9	a'	16.0	8	6.0	37.5
b	19.0	8	b'	23.5	10	—4.5	—19.1
a	24.0	8	a'	18.5	7	5.5	29.7
b	18.0	6	b'	12.5	4	5.5	44.4
a	22.0	8	a'	20.5	5	1.5	7.3
b	27.5	10	b'	25.0	12	2.5	10.0
a	19.0	7	a'	16.5	6	2.5	15.2
b	27.0	9	b'	29.0	11	—2.0	—6.9
a	37.5	10	a'	24.0	9	13.5	56.2
b	22.0	9	b'	13.0	8	9.0	69.2
*a	10.0	2	*a'	9.0	4	1.0	11.1
b	22.5	9	b'	21.0	9	1.5	7.1
*a	16.0	9	*a'	10.0	9	6.0	60.0
b	20.0	6	b'	27.0	9	—7.0	—25.9
a	23.0	13	a'	19.5	5	3.5	17.9
b	18.5	8	b'	19.0	10	—0.5	—2.6
a	19.5	7	a'	14.5	6	5.0	34.5
b	28.0	7	b'	21.5	8	6.5	30.2
a	30.5	10	a'	27.5	9	2.5	9.1
b	25.5	9	b'	18.0	4	7.5	41.7
a	32.0	10	a'	23.5	7	8.5	36.2
b	29.0	9	b'	24.5	6	4.5	18.3
a	28.0	7	a'	22.5	6	5.5	24.4
b	27.0	7	b'	28.0	10	—1.0	—10.0
a	23.0	8	a'	24.5	7	—1.5	—6.1
b	31.0	7	b'	21.5	6	9.5	44.4
a	26.0	7	a'	19.5	8	6.5	33.3
b	25.5	12	b'	18.0	5	7.5	41.7
a	22.0	9	a'	14.0	5	8.0	57.1
b	19.0	7	b'	19.5	6	—0.5	—2.6
a	16.5	7	a'	10.5	5	6.0	57.1
b	21.0	7	b'	16.0	10	5.0	31.2
a	23.0	9	a'	25.0	10	—2.0	—8.0
b	22.5	11	b'	22.0	9	—0.5	—2.3
a	31.5	13	a'	25.5	6	6.0	23.5
b	30.5	9	b'	25.5	8	5.0	19.6
a	26.5	5	a'	28.5	10	—2.0	—7.0
b	23.5	6	b'	20.0	5	3.5	17.5
a	30.5	6	a'	26.0	6	4.5	17.3
b	34.5	11	b'	30.0	6	4.5	15.0
a	40.0	8	a'	25.0	9	15.0	60.0

TABLE I (*continued*).

Exterior Plants.			Interior Plants.			Difference in yield ($a - a'$ or $b - b'$).	Percentage of increase or decrease in yield ($\frac{a - a'}{a'}$ or $\frac{b - b'}{b'}$).
Plant.	Weight of tubers.	Number of tubers.	Plant.	Weight of tubers.	Number of tubers.		
	Oz.			Oz.		Oz.	Per ct.
b	22.0	8	b'	27.5	10	—5.5	—20.0
a	28.0	6	a'	31.0	9	—3.0	—9.7
b	27.5	7	b'	24.0	5	3.5	14.6
a	41.0	17	a'	29.0	12	12.0	41.4
b	25.0	7	b'	25.0	7	0.0	0.0
a	14.5	6	a'	18.5	5	—4.0	—21.6
b	17.5	9	b'	12.5	5	5.0	40.0
a	24.5	7	a'	19.0	6	5.5	28.9
b	16.5	6	b'	20.0	5	—3.5	—17.5
*a	10.5	8	*a'	6.5	4	4.0	61.5
b	22.0	5	b'	16.5	4	5.5	33.3
a	17.5	9	a'	18.0	7	—0.5	—2.8
b	32.0	8	b'	22.0	7	10.0	45.5
a	23.5	6	a'	21.0	7	2.5	11.9
b	28.0	6	b	20.5	8	7.5	36.6
a	30.0	5	a'	19.5	6	10.5	53.8
b	29.0	10	b'	27.5	9	1.5	5.5
a	29.5	11	a'	21.5	10	8.0	37.2
b	28.0	11	b'	21.5	10	6.5	30.2
a	21.0	5	a'	18.5	5	2.5	13.5
b	21.5	6	b'	19.5	6	2.0	10.3
a	29.5	8	a'	19.0	5	10.5	55.3
b	23.0	7	b'	14.5	8	8.5	58.8
a	18.0	8	a'	16.0	5	2.0	12.5
*b	6.5	2	*b'	5.0	4	1.5	30.0
a	23.5	6	a'	23.5	5	0.0	0.0
b	22.0	9	b'	19.0	6	3.0	15.8
a	30.5	12	a'	25.5	9	5.0	19.6
b	16.0	7	b'	17.5	8	—1.5	—8.6
a	29.0	11	a'	24.5	12	4.5	18.4
b	23.0	7	b'	21.0	7	2.0	9.5
a	29.0	9	a'	27.0	4	2.0	7.4
b	33.0	9	b'	23.0	7	10.0	43.5
a	26.0	7	a'	24.0	7	2.0	8.3
b	34.5	7	b'	22.0	6	12.5	56.8
a	23.5	9	a'	19.0	7	4.5	23.7
b	34.0	10	b'	26.0	8	8.0	30.8
a	30.0	8	a'	24.5	10	5.5	22 4
b	46.0	12	b'	25.0	9	21.0	84.0
*a	12.0	9	*a'	12.0	7	0.0	0.0
b	35.5	9	b'	41.5	12	—6.0	—14.5
a	29.0	10	a'	18.5	5	10.5	56.8

TABLE I (*continued*).

Exterior Plants.			Interior Plants.			Difference in yield ($a - a'$ or $b - b'$).	Percentage of increase or decrease in yield ($\frac{a - a'}{a'}$ or $\frac{b - b'}{b'}$).
Plant.	Weight of tubers.	Number of tubers.	Plant.	Weight of tubers.	Number of tubers.		
	Oz.			Oz.		Oz.	Per ct.
b	18.5	5	b'	13.0	5	5.5	42.3
a	17.0	6	a'	13.5	6	3.5	25.9
b	25.0	9	b'	32.0	8	—7.0	—21.9
a	26.5	8	a'	14.0	4	12.5	89.3
b	15.5	5	b'	15.0	4	0.5	3.3
a	18.5	9	a'	14.0	6	4.5	32.1
*b	5.0	4	*b'	6.0	5	—1.0	—16.7
a	28.5	5	a'	16.5	6	12.0	72.7
b	25.0	5	b'	22.5	6	2.5	11.1
a	22.0	7	a'	16.0	8	6.0	37.5
b	28.0	7	b'	29.0	7	—1.0	—3.4
a	16.0	4	a'	16.0	4	0.0	0.0
b	19.0	6	b'	18.5	6	0.5	2.7
a	20.0	6	a'	12.5	4	7.5	60.0
*b	4.5	4	*b'	6.5	5	—2.0	—30.8
a	16.5	6	a'	15.5	6	1.0	6.5
b	23.5	6	b'	20.0	6	3.5	17.5
a	19.0	6	a'	20.5	4	—1.5	—7.3
b	18.5	6	b'	17.0	6	1.5	8.8
a	20.0	5	a'	22.5	5	—2.5	—11.1
a	22.5	10	a'	13.5	6	9.0	66.7
b	26.0	10	b'	18.0	7	8.0	44.4
a	30.0	11	a'	17.0	7	13.0	76.5
b	23.5	9	b'	20.0	10	3.5	17.5
a	27.0	9	a'	21.0	7	6.0	28.6
b	22.5	7	b'	23.5	4	—1.0	—4.3
a	28.5	8	a'	21.5	6	7.0	32.6
b	26.5	10	b'	24.5	11	2.0	8.2
a	45.0	12	a'	32.0	13	13.0	40.6
b	38.0	11	b'	24.5	10	13.5	55.1
a	35.5	11	a'	29.0	8	6.5	22.4
b	32.0	10	b'	20.5	6	11.5	56.1
a	42.5	12	a'	24.0	11	18.5	77.1
b	45.0	10	b'	22.5	10	22.5	100.0
a	22.5	10	a'	19.0	10	3.5	18.4
b	22.0	7	b'	22.0	8	0.0	0.0
a	22.0	7	a'	17.5	5	4.5	25.7
b	22.5	8	b'	16.0	6	6.5	40.6
a	23.0	11	a'	19.0	8	4.0	21.1
b	18.0	6	b'	17.0	5	1.0	5.9
a	18.0	7	a'	13.5	10	4.5	33.3
*b	6.0	3	*b'	9.0	4	—3.0	—33.3

TABLE I (*continued*).

Exterior Plants.			Interior Plants.			Difference in yield ($a - a'$ or $b - b'$).	Percentage of increase or decrease in yield ($\frac{a - a'}{a'}$ or $\frac{b - b'}{b'}$).
Plant.	Weight of tubers.	Number of tubers.	Plant.	Weight of tubers.	Number of tubers.		
	Oz.			*Oz.*		*Oz.*	*Per ct.*
a	19.0	8	*a'*	15.0	5	4.0	26.7
b	21.0	6	*b'*	16.0	6	5.0	31.2
a	19.0	7	*a'*	15.5	5	3.5	22.6
b	25.0	10	*b'*	21.5	7	3.5	16.3
a	31.5	10	*a'*	27.5	9	4.0	14.5
b	32.0	9	*b'*	34.0	8	—2.0	—5.9
a	24.0	8	*a'*	18.5	6	5.5	29.7
b	18.5	9	*b'*	16.0	6	2.5	15.6
a	14.0	6	*a'*	18.5	6	—4.5	—24.3
b	23.0	6	*b'*	17.0	8	6.0	35.3
a	25.5	6	*a'*	19.5	6	6.0	30.8
b	13.0	4	*b'*	11.0	4	2.0	18.2
a	33.0	9	*a'*	21.0	6	12.0	57.1
b	22.5	15	*b'*	16.0	8	6.5	40.6
a	42.5	12	*a'*	30.0	8	12.5	41.7
b	20.0	9	*b'*	20.0	8	0.0	0.0
a	28.5	4	*a'*	14.0	2	14.5	103.6
b	25.5	8	*b'*	19.0	7	6.5	34.2
a	39.5	13	*a'*	27.5	10	12.0	43.6
**b*	16.5	9	**b'*	8.0	6	8.5	106.2
a	45.5	14	*a'*	27.5	13	18.0	65.6
b	33.0	13	*b'*	23.0	8	10.0	43.5
a	32.0	9	*a'*	31.5	7	0.5	1.6
b	32.0	9	*b'*	27.0	11	5.0	18.5
a	31.0	8	*a'*	21.0	6	10.0	47.6
b	28.5	6	*b'*	27.0	7	1.5	5.6
a	48.5	16	*a'*	30.0	10	18.5	61.7
b	34.5	11	*b'*	22.0	6	12.5	56.8
a	24.5	9	*a'*	18.0	5	6.5	36.1
b	16.0	4	*b'*	15.0	4	1.0	6.7
a	16.0	6	*a'*	19.5	8	—3.5	—17.9
b	15.0	7	*b'*	10.0	4	5.0	50.0
a	10.5	3	*a'*	16.0	6	—5.5	—34.4
b	18.0	7	*b'*	13.5	5	4.5	33.3
a	24.0	12	*a'*	20.5	9	3.5	17.1
b	15.0	4	*b'*	10.0	4	5.0	50.0
a	13.5	9	*a'*	14.0	6	—0.5	—3.6
b	22.5	8	*b'*	18.5	8	4.0	21.6
a	20.5	7	*a'*	15.0	7	5.5	36.7
**b*	15.5	8	**b'*	10.0	4	5.5	55.0
a	20.0	6	*a'*	18.0	8	2.0	11.1
b	26.0	10	*b'*	19.5	7	6.5	33.3

TABLE I (*continued*).

Exterior Plants.			Interior Plants.			Difference in yield ($a - a'$ or $b - b'$).	Percentage of increase or decrease in yield ($\frac{a-a'}{a'}$ or $\frac{b-b'}{b'}$).
Plant.	Weight of tubers.	Number of tubers.	Plant.	Weight of tubers.	Number of tubers.		
	Oz.			*Oz.*		*Oz.*	*Per ct.*
a	18.0	7	*a'*	18.5	7	—0.5	—2.7
b	24.0	8	*b'*	20.0	10	4.0	20.0
a	14.0	4	*a'*	20.0	4	—6.0	—30.0
b	17.0	9	*b'*	18.5	9	—1.5	—8.1
a	26.5	11	*a'*	24.5	10	2.0	8.2
b	16.5	4	*b'*	20.5	7	—4.0	—19.5
**a*	9.0	4	**a'*	10.5	3	—1.5	—14.3
b	29.0	6	*b'*	24.0	7	5.0	20.8
b	18.5	9	*b'*	35.0	11	—16.5	—47.1
a	40.0	13	*a'*	24.5	11	15.5	63.3
b	31.5	11	*b'*	30.0	13	1.5	5.0
a	19.0	14	*a'*	33.0	13	—14.0	—42.4
b	35.5	12	*b'*	20.5	7	15.0	73.2
a	36.0	10	*a'*	28.5	9	7.5	26.3
b	27.5	8	*b'*	26.5	8	1.0	3.8
a	37.5	8	*a'*	31.0	8	6.5	21.0
b	36.0	9	*b'*	24.5	7	11.5	46.9
a	29.0	8	*a'*	21.0	9	8.0	38.1
b	23.5	8	*b'*	27.5	9	—4.0	—14.5
a	32.5	11	*a'*	24.5	8	8.0	32.7
b	35.0	11	*b'*	30.0	9	5.0	16.7
a	24.0	6	*a'*	23.0	7	1.0	4.3
b	32.0	8	*b'*	19.0	10	13.0	68.4
a	21.0	12	*a'*	18.5	7	2.5	13.5
**b*	10.5	4	**b'*	7.5	5	3.0	40.0
a	23.0	7	*a'*	17.5	8	5.5	31.4
b	19.5	7	*b'*	20.5	7	—1.0	—4.9
a	24.0	13	*a'*	16.5	10	7.5	45.5
b	23.5	7	*b'*	19.5	6	4.0	20.5
a	23.0	6	*a'*	18.5	7	4.5	24.3
b	27.0	8	*b'*	20.0	11	7.0	35.0
a	22.0	8	*a'*	21.5	5	0.5	2.3
**b*	7.0	6	**b'*	5.5	3	1.5	27.3
a	18.0	8	*a'*	20.0	7	—2.0	—10.0
b	20.0	8	*b'*	22.5	6	—2.5	—11.1
a	24.0	9	*a'*	21.0	9	3.0	14.3
b	21.5	13	*b'*	20.5	6	1.0	4.9
**a*	17.5	11	**a'*	14.5	13	3.0	20.7
b	22.0	7	*b'*	22.5	6	—0.5	—2.2
a	29.0	10	*a'*	20.0	6	9.0	45.0
**b*	7.0	3	**b'*	7.5	4	—0.5	—6.7
a	22.0	11	*a'*	18.5	10	3.5	18.9

TABLE I (*continued*).

Exterior Plants.			Interior Plants.			Difference in yield ($a-a'$ or $b-b'$).	Percentage of increase or decrease in yield ($\frac{a-a'}{a'}$ or $\frac{b-b'}{b'}$).
Plants.	Weight of tubers.	Number of tubers.	Plant.	Weight of tubers.	Number of tubers.		
	Oz.			Oz.		Oz.	Per ct.
b	25.0	11	b'	17.5	7	7.5	42.9
a	28.0	7	a'	26.0	10	2.0	7.7
b	31.0	7	b'	20.0	8	11.0	55.0
a	34.5	9	a'	26.0	9	8.5	32.7
b	23.0	10	b'	26.0	8	—3.0	—11.5
a	31.0	15	a'	21.5	7	9.5	44.2
b	32.0	10	b'	27.0	6	5.0	18.5
a	31.0	10	a'	21.5	6	9.5	44.2
b	40.0	11	b'	23.0	7	17.0	73.9
a	31.0	7	a'	21.0	9	10.0	47.6
b	23.0	6	b'	20.5	6	2.5	12.2
a	36.0	14	a'	24.5	6	11.5	46.9
b	37.5	10	b'	19.0	5	18.5	97.4
a	32.5	11	a'	26.0	7	6.5	25.0
b	36.0	9	b'	30.0	10	6.0	20.0
*a	11.0	6	*a'	11.0	4	0.0	0.0
*b	8.5	6	*b'	10.5	7	—2.0	—19.0
a	27.5	11	a'	24.0	8	3.5	14.6
b	26.5	7	b'	24.0	9	2.5	10.4
a	24.0	8	a'	24.0	7	0.0	0.0
b	25.5	10	b'	26.0	11	—0.5	—1.9
a	24.5	6	a'	20.5	3	4.0	19.5
b	21.5	7	b'	20.0	6	1.5	7.5
a	39.0	13	a'	32.0	8	7.0	21.9
*b	20.5	7	*b'	14.0	8	6.5	46.4
*a	8.0	5	*a'	9.5	4	—1.5	—15.8
b	23.5	9	b'	28.0	8	—4.5	—16.1
a	27.5	8	a'	22.5	8	5.0	22.2
b	26.0	7	b'	16.0	5	10.0	62.5
a	40.0	8	a'	23.5	9	16.5	70.2
*b	9.5	5	*b'	6.0	3	3.5	58.3
a	29.0	11	a'	28.5	7	0.5	1.8
a	23.0	6	a'	18.5	5	4.5	24.3
b	30.5	7	b'	23.0	7	7.5	32.6
a	30.5	7	a'	27.0	8	3.5	13.0
a	28.5	7	a'	20.5	6	8.0	39.0
b	26.5	6	b'	29.5	5	—3.0	—10.2
a	31.0	9	a'	23.0	7	8.0	34.8
b	28.0	9	b'	24.0	5	4.0	16.7
a	28.5	7	a'	17.5	9	11.0	62.9
b	24.0	6	b'	20.5	6	3.5	17.1
a	24.5	10	a'	18.5	7	6.0	32.4

TABLE I (*continued*).

Exterior Plants.			Interior Plants.			Difference in yield ($a-a'$ or $b-b'$).	Percentage of increase or decrease in yield ($\frac{a-a'}{a'}$ or $\frac{b-b'}{b'}$).
Plant.	Weight of tubers.	Number of tubers.	Plant.	Weight of tubers.	Number of tubers.		
	Oz.			*Oz.*		*Oz.*	*Per ct.*
b	16.5	5	b'	24.5	8	—8.0	—32.7
a	24.0	8	a'	19.0	8	5.0	26.3
a	35.5	6	a'	19.0	5	16.5	86.8
b	25.5	8	b'	16.5	7	9.0	54.5
a	24.0	10	a'	23.0	12	1.0	4.3
b	22.5	10	b'	22.5	12	0.0	0.0
a	24.0	8	a'	19.0	4	5.0	26.3
b	29.5	10	b'	25.0	7	4.5	18.0
a	23.0	10	a'	23.0	9	0.0	0.0
b	30.0	6	b'	23.0	5	7.0	30.4
a	30.0	8	a'	21.0	5	9.0	42.9
b	33.0	13	b'	23.0	6	10.0	43.5
a	27.5	9	a'	26.5	8	1.0	3.8
*a	13.5	9	*b'	12.0	3	1.5	12.5
*a	10.5	7	*a'	10.0	7	0.5	5.0
b	30.0	9	b'	28.0	9	2.0	7.1
a	19.5	8	a'	16.5	8	3.0	18.2
b	29.0	8	b'	25.5	8	3.5	13.8
a	38.5	13	a'	29.5	8	9.0	30.5
b	31.0	6	b'	24.0	7	7.0	29.2
a	29.5	10	a'	27.0	10	2.5	9.3
b	20.5	7	b'	21.5	11	—1.0	—4.7
a	22.0	6	a'	22.0	10	0.0	0.0
b	27.5	6	b'	20.0	9	7.5	37.5
a	28.0	10	a'	23.0	6	5.0	21.7
b	27.5	8	b'	25.0	6	2.5	10.0
a	19.0	3	a'	8.5	3	10.5	123.5
b	30.0	7	b'	27.5	7	2.5	9.1
a	22.0	8	a'	25.0	6	—3.0	—12.0
b	23.0	7	b'	21.5	5	1.5	7.0
a	32.0	8	a'	23.5	6	8.5	36.2
b	17.0	7	b'	14.0	8	3.0	21.4
*a	6.0	4	*a'	3.5	4	2.5	71.4
b	28.0	9	b'	28.5	8	—0.5	—1.8
a	23.0	8	a'	31.0	8	—8.0	—25.8
*b	13.5	7	*b'	8.0	4	5.5	68.7
a	21.0	5	a'	22.0	5	—1.0	—4.5
b	34.5	8	b'	24.0	11	10.5	43.7
*a	5.0	4	*a'	9.0	4	—4.0	—44.4
b	23.0	9	b'	21.5	6	1.5	7.0
a	25.0	8	a'	24.0	9	1.0	4.2
b	20.5	6	b'	20.0	7	0.5	2.5

TABLE I (*concluded*).

Exterior Plants.			Interior Plants.			Difference in yield ($a - a'$ or $b - b'$).	Percentage of increase or decrease in yield ($\frac{a - a'}{a'}$ or $\frac{b - b'}{b'}$).
Plant.	Weight of tubers.	Number of tubers.	Plant.	Weight of tubers.	Number of tubers.		
	Oz.			*Oz.*		*Oz.*	*Per ct.*
a	31.0	11	a'	21.0	5	10.0	47.6
b	28.5	9	b'	28.5	8	0.0	0.0
a	26.0	7	a'	26.5	5	—0.5	—1.9
b	25.0	6	b'	22.0	8	3.0	13.6
a	29.5	9	a'	22.0	8	7.5	34.1
b	34.5	13	b'	28.0	8	6.5	23.2
a	30.0	12	a'	27.5	8	2.5	9.1
b	34.5	8	b'	20.0	6	14.5	72.5
*a	16.0	6	*a'	13.0	5	3.0	23.1
b	27.5	8	b'	29.0	7	—1.5	—5.2
a	42.5	8	a'	26.0	7	16.5	63.5
b	36.5	9	b'	20.0	9	16.5	82.5
a	39.0	9	a'	25.5	8	13.5	52.9
b	32.0	9	b'	26.5	8	5.5	20.8
a	35.5	9	a'	26.0	10	9.5	36.5
b	29.0	8	b'	23.5	5	5.5	23.4
*a	13.0	6	*a'	13.0	5	0.0	0.0
b	37.0	13	b'	30.0	8	7.0	23.3
*a	14.5	8	*a'	9.0	7	5.5	61.1
b	31.5	11	b'	40.5	8	—9.0	—22.2
a	37.0	9	a'	36.0	8	1.0	2.8
b	27.5	9	b'	28.0	6	—0.5	—1.8
a	24.5	8	a'	25.0	9	—0.5	—2.0
a	22.0	7	a'	21.0	6	1.0	4.8
b	26.5	11	b'	22.0	7	4.5	20.5
a	13.0	6	a'	13.0	5	0.0	0.0
b	19.0	8	b'	24.0	8	—5.0	—20.8
a	29.5	8	a'	22.5	7	7.0	31.1
a	37.5	6	a'	27.5	7	10.0	36.4
*b	6.5	4	*b'	3.5	3	3.0	85.7
a	22.0	7	a'	13.5	6	8.5	63.0
b	21.0	9	b'	25.0	8	—4.0	—16.0
a	30.0	13	a'	25.5	7	4.5	17.6
b	33.0	8	b'	23.5	7	9.5	40.4

THE BULK RECORD.

The bulk record is shown in Table II. Here are given the number and weight of tubers produced by the *a* and *b* (exterior) plants and by the *a′* and *b′* (interior) plants. The yield of normal and leaf roll plants in each of these two groups has been given separately and combined. In each division the tubers have been sorted into three classes, according to their size, and the number and weight of the tubers of each class recorded.

In Table III the data given in Table II have been reduced to averages per plant.

TABLE II.— YIELDS IN MISSING HILL EXPERIMENT: THE BULK RECORD.

Condition of health	Kind of plants*	Number of plants	Tubers over 2 Oz.		Tubers between 1 and 2 Oz.		Tubers under 1 Oz.		Total yield: Tubers of all sizes.	
			Number.	Weight.	Number.	Weight.	Number.	Weight.	Number.	Weight.
				Oz.		*Oz.*		*Oz.*		*Oz.*
Normal.	*a* and *b*	321	1,885	7,452.5	543	815.0	233	135.5	2,661	8,403.0
	a′ and *b′*	321	1,599	6,060.0	529	815.5	173	94.0	2,301	6,969.5
Leaf roll.	*a* and *b*	30	82	237.5	49	66.5	46	26.0	177	330.0
	a′ and *b′*	30	57	168.5	54	74.5	40	22.5	151	265.5
Normal and leaf roll combined.	*a* and *b*	351	1,967	7,600.0	592	881.5	279	161.5	2,838	8,733.0
	a′ and *b′*	351	1,656	6,228.5	583	890.0	213	116.5	2,452	7,235.0

* Plants adjoining blank spaces are designated *a* and *b* plants; those not adjoining blank spaces, *a′* and *b′* plants.

TABLE III.— AVERAGE YIELD PER PLANT IN MISSING HILL EXPERIMENT.*

Condition of health.	Kind of plants	Number of plants	Tubers over 2 Oz.		Tubers between 1 and 2 Oz.		Tubers under 1 Oz.		Total yield: Tubers of all sizes.	
			Aver. no. per plant.	Aver. wt. per plant.	Aver. no. per plant.	Aver. wt. per plant.	Aver. no. per plant.	Aver. wt. per plant.	Aver. no. per plant.	Aver. wt. per plant.
				Oz.		*Oz.*		*Oz.*		*Oz.*
Normal.	*a* and *b*	321	5.87	23.22	1.69	2.54	0.73	0.42	8.29	26.18
	a′ and *b′*	321	4.98	18.88	1.65	2.54	0.54	0.29	7.17	21.71
Leaf roll.	*a* and *b*	30	2.73	7.92	1.63	2.22	1.53	0.87	5.90	11.00
	a′ and *b′*	30	1.90	5.62	1.80	2.48	1.33	0.75	5.03	8.85
Normal and leaf roll combined.	*a* and *b*	351	5.60	21.91	1.69	2.51	0.79	0.46	8.08	24.88
	a′ and *b′*	351	4.72	17.74	1.66	2.54	0.61	0.33	6.99	20.61

* Calculated from data in Table II.

HILL RECORD AND BULK RECORD COMPARED.

It will be observed that by comparing the figures in the hill record with those in the bulk record the accuracy of the work can be tested. For this purpose Tables IV and V have been prepared. The former contains a summary of the hill and bulk records of the experiment proper, while the latter is a summary of the hill and bulk records of the check.

TABLE IV.— SUMMARY OF YIELDS IN MISSING HILL EXPERIMENT: COUNTS AND WEIGHTS OF HILL RECORD AND BULK RECORD COMPARED.

HILL RECORD.				BULK RECORD.			
a and *b* plants.* (Exterior plants.)		*a'* and *b'* plants.† (Interior plants.)		*a* and *b* plants.* (Exterior plants.)		*a'* and *b'* plants.† (Interior plants.)	
Number of tubers.	Total weight.	Number of tubers.	Total weight.	Number of tubers.	Total weight.	Number of tubers.	Total weight.
	Oz.		*Oz.*		*Oz.*		*Oz.*
2825	8713	2455	7206	2838	8733	2452	7235

* Plants adjoining blank spaces.
† Plants not adjoining blank spaces.

TABLE V.— SUMMARY OF YIELDS OF CHECK IN MISSING HILL EXPERIMENT: COUNTS AND WEIGHTS OF HILL RECORD AND BULK RECORD COMPARED.

HILL RECORD.				BULK RECORD.			
c plants.		*c'* plants.		*c* plants.		*c'* plants.	
Number of tubers.	Total weight.	Number of tubers.	Total weight.	Number of tubers.	Total weight.	Number of tubers.	Total weight.
	Oz.		*Oz.*		*Oz.*		*Oz.*
601	1835	641	1941	598	1784.5	643	1888.5

In Table IV it will be observed that, in the experiment proper, the counts and weights of the hill record agree fairly well with those of the bulk record. The difference in weight is only 20 ounces for the exterior plants and 29 ounces for the interior plants, or less than one-third of one per ct. in each case. While this discrepancy is too large to be entirely ignored, it does not invalidate the data.

From Table V it will be seen that the counts in the hill and bulk records of the check agree well, but the weights are somewhat discordant, the difference being equal to 2.7 per ct. of the weight shown by the hill record.

DISCUSSION OF RESULTS.

THE CHECK.

Notwithstanding the precautions taken to eliminate everything which might cause inequality between the members of a pair of plants from the same tuber, there was much variation in yield. The difference in yield varied from 0 to 13 ounces per pair, the average for 85 pairs of plants being 4.51 ounces or 20.3 per ct. of the average yield per plant.

Another and better form of statement is that the difference in yield varied, for different pairs, from 0 to 66.7 per ct. of the mean yield of the two plants of the pair, and that the mean difference was 20.7 ± 1.1 per ct.[2] Yet another way of expressing it is to say that the coefficient of variability was 74.9 per ct.[3]

Strange to say, the c' plants outyielded their mates, the c plants, by 1.24 ounces on the average. While it is conceivable that unrecognized factors may cause some variation in yield, it is to be expected that such variation would disappear when averages are made. To be sure, 85 pairs is a rather small number upon which to base an average. Also, there is the possibility that the yields were inaccurately determined. However, it should be noted that practically the same difference is shown by the hill record and the bulk record; and the greater number of tubers from the c' plants tends to support the belief that there was actually a greater weight of tubers from the c' plants.

[2] Calculated in the following manner: $\frac{c - c'}{\frac{1}{2}(c + c')}$ or $\frac{c' - c}{\frac{1}{2}(c + c')}$ = percentage of difference in individual pairs.

$$\frac{\text{Sum of individual percentages of difference}}{\text{Total number of pairs}} = \text{mean difference} = 20.7 \text{ per ct.}$$

Probable error of the mean $= \pm 0.6745\sqrt{\frac{\Sigma d^2}{n^2}}$ when Σd^2 represents the sum of the squares of the deviations of the individual variants from the mean and n the number of variants.

[3] Calculated by the formula, $CV = \frac{\text{standard deviation}}{\text{mean}} \times 100$ or $\frac{100\sigma}{M}$, the formula for standard deviation being $\sigma = \sqrt{\frac{\Sigma d^2}{n}}$.

Whatever the cause, the failure to secure more nearly equal yields from plants under supposedly parallel conditions is noteworthy. It shows that there are factors having a very important bearing on the yield of potatoes which are either unknown or not estimated at their proper value. In experimental work it raises the question of the validity of conclusions based upon the yield of a small number of potato plants. Also, it emphasizes the importance of having proper checks, and giving careful attention to all of the details of culture in plat experiments with potatoes. Not only should these matters be carefully attended to in the field, but a full account of them should accompany the publication of the results of the experiment. The practice of publishing the results of plat experiments with potatoes without giving full details concerning checks and the size, shape, arrangement and treatment of plats is to be condemned. Lacking information on these points, the reader is unable to interpret the results of an experiment or judge of the trustworthiness of the conclusions drawn by the writer.

EXTERIOR PLANTS VERSUS INTERIOR PLANTS.

From even a casual examination of the data it is evident that the exterior plants outyielded the interior ones; but the exact amount of the difference depends upon the method of calculating it, and whether one uses the data in the hill record or those in the bulk record.

The simple, natural method of calculation would be as follows: Find the average yield of the interior plants by dividing their total yield by the number of plants (351). In a similar manner find the average yield of the exterior plants. Now, by subtracting the average yield of the interior plants from the average yield of the exterior plants, the difference in yield in ounces per plant is obtained, and this divided by the average yield of the interior plants gives the average percentage of increase in yield shown by the exterior plants.

But the percentage so obtained will be only approximately correct. It will not be strictly correct because no one of the exterior plants is strictly comparable with any other plant except its interior mate. Plants produced by seed-pieces from different tubers are not comparable, particularly if the seed-pieces were unequal in size. Plants having a different number of stalks are not comparable. Plants affected with leaf roll are not comparable with healthy plants.

In other words, each pair of plants (one interior and the other exterior) from the two halves of the same seed-tuber constitutes a separate experiment. The difference in yield between the two members of a pair must be expressed in percentage of the yield of the interior plant before it can be compared with the difference in the yield of any other pair.

The proper method of calculation, then, is as follows: First, for each pair of plants, find what per ct. the difference in yield is of the yield of the interior plant by dividing the former by the latter. If the difference is in favor of the exterior plant give it a plus sign; if in favor of the interior plant, a minus sign. Second, take the algebraic sum of the percentages so found, and divide by the total number of pairs (351). The quotient is the mean percentage of increase.

In 14 pairs the exterior plant gave the same yield as its interior mate. In 272 pairs the exterior plant outyielded the interior one, the greatest difference being 22.5 ounces. In 65 pairs the interior plant outyielded the exterior one, the greatest difference being 16.5 ounces. Thus the "increase in yield" of the exterior plants over the interior plants varied from —16.5 ounces to +22.5 ounces.

Applying the first method of calculation and using the data of the hill record,[4] the exterior plants outyielded the interior ones by 4.3 ounces per plant. This is 20.9 per ct. of the average yield of the interior plants.

Applying the second and more exact method of calculation, the "increase" is found to vary from —47.1 per ct. to +141.7 per ct., the average, or mean, being +23.2 per ct. with a probable error of ±1.03.[5] The frequency with which the different percentages of increase occurred is shown graphically in Figure 8.

Accordingly, the answer given by the experiment is that, under conditions such as obtained in this experiment, the adjoining plants on either side of a single missing hill together make up 46.4 per ct. of the loss in total yield. This agrees well with the conclusion of Fitch and Bennett, namely, "That the hills on either side of a skip make up one-half the loss." The agreement might have been yet

[4] Using the bulk record, the average difference in yield is 4.27 ounces or 20.7 per ct.

[5] The probable error of a mean is calculated by the formula,

$$E_m = \pm 0.6745 \sqrt{\frac{\Sigma d^2}{n^2}}.$$

closer, perhaps, had the experiment been so planned as to take into account the influence of the missing hills upon the second plant on either side. Fitch and Bennett assert that skips in some varieties affect the yield of the second plant on either side.[6]

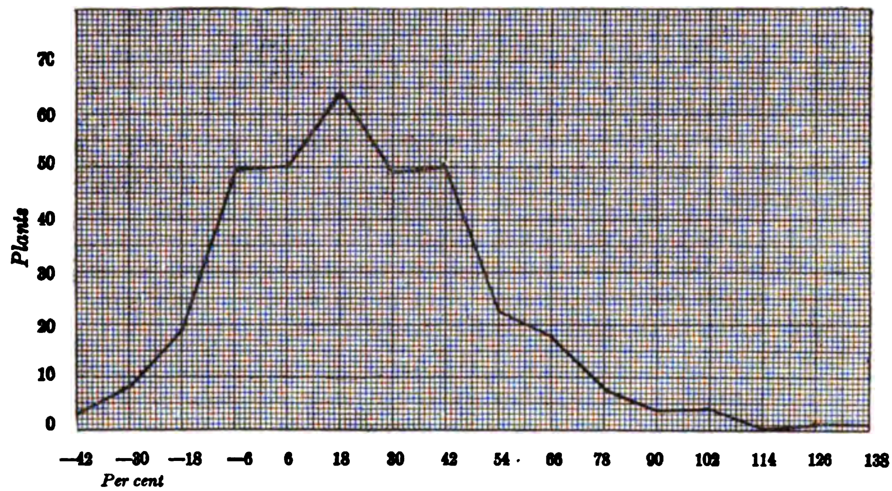

Fig. 8.— Curve showing distribution of percentages of increase and decrease in the yield of 351 potato plants under the influence of adjoining missing hills: a graphic representation of the data in Table I. A minus (—) sign indicates a decrease in yield. Mean, 23.2±1.03 per ct.; standard deviation, 28.39±0.72 per ct.; coefficient of variability, 122.36±5.11 per ct.

So far, only total yield has been considered; but it would be of interest to know how the yield of marketable tubers was affected. Unfortunately, it seemed impracticable to grade the product of each plant separately. Hence, the only data we have on the yield

[6] Fitch and Bennett, *Loc. cit.* While this may be true in thick planting, it is doubtful if it occurs where the plants are 15 inches apart. Probably, skips of more than one hill affect the yield of adjoining plants no more than do skips of a single hill. But we have no data bearing directly upon these points. In the writer's judgment, the data given by Fitch and Bennett do not warrant the conclusion which they draw. In fact, it may be said that their data have little value for any purpose because they are based upon plants whose history is unknown. Undoubtedly, the plants whose yields they compared were from different seed-tubers and from seed-pieces of diverse sizes — two factors which influence yield profoundly. Also, the data were from nine different varieties (some early and some late) in four localities, and planted different distances apart in the row. The number of plants of any one kind admitting of close comparison was so small as to make it impossible to secure dependable averages. General averages from such miscellaneous data mean nothing.

of tubers of different sizes are those shown in the bulk record. (Table II.) While, for reasons previously stated, these data do not answer our purpose perfectly, they enable us to draw conclusions which approximate the truth. According to the bulk record 88 per ct. of the total yield of exterior plants was of marketable size, that is, in tubers weighing over two ounces each; while only 86 per ct. of the total yield of interior plants was of marketable size. Apparently, the exterior plants made a slightly better showing in marketable tubers than in total yield.

The exterior plants produced not only a greater weight of tubers, but, also, a greater number of tubers. To be sure, this was not invariably the case, but it was plainly true of the average. In 57 pairs of plants the two members produced the same number of tubers; in 89 pairs the interior plant produced the greater number of tubers; while in 205 pairs the exterior plant produced the greater number. By applying to the data of the hill record the two methods of calculation described on pages 63 and 64 it may be shown by the first method that the exterior plants yielded, on the average, 8.05 tubers per plant while the interior plants gave an average yield of 6.99 tubers per plant. The difference, expressed in percentage of the average yield of the interior plants, is 15.16 per ct. By the better method of calculation, the exterior plants produced 20.7 per ct. more tubers per plant than did the interior ones.

According to the bulk record, the exterior plants produced, on the average, 5.6 marketable tubers having an average weight of 3.91 ounces. The interior plants produced 4.7 marketable tubers having an average weight of 3.76 ounces.

EFFECT OF LEAF ROLL.

In the above comparison of the yields of exterior plants, no distinction has been made between normal and leaf roll plants. The data used were taken from 351 pairs of plants of which 321 pairs were normal, and 30 pairs severely affected with leaf roll.

It may be argued that the leaf roll plants should have been excluded from consideration because they were much smaller than the normal plants and, presumably, their roots did not extend as far as those of normal plants. Hence, the yield of leaf roll plants would be less affected by missing hills. But the data obtained in the experiment do not support this view. They appear to prove, on the contrary,

that the yield of leaf roll plants is more affected than that of normal plants. Leaf roll plants adjoining missing hills outyielded their mates by 26.8 per ct. on the average, while exterior normal plants outyielded their mates by only 22.8 per ct. on the average.

It is difficult to account satisfactorily for this apparently greater response of the leaf roll plants. It can scarcely have been due to errors in recording the data, because the counts and weights of the bulk record agreed quite closely with those of the hill record. Since the number of leaf roll plants was small (30 pairs), it may have been merely the result of chance.[7]

Besides the direct method given above there is, also, an indirect method of proving that the yield of leaf roll plants was affected by adjoining missing hills. If the roots of leaf roll plants do not extend far enough to derive benefit from adjoining blank spaces, they cannot extend far enough to affect the yield of adjoining normal plants. Hence, a pair of normal plants adjoined on one side by a missing hill and on the other by a leaf roll plant should give the same yield. That is to say, leaf roll plants would have practically the same effect as missing hills. Let us see if the data bear this out.

It will be remembered that the potato plants were in groups of four (two pairs) with a blank space on either side. Two pairs of leaf roll plants occurred in the same group, but each of the remaining 28 pairs was grouped with a pair of normal plants. Accordingly, there were 28 pairs of normal plants one member of which adjoined a missing hill, while the other adjoined a leaf roll plant. It is an interesting fact that the yield of the plants adjoining blank spaces was, on the average, 15.5 ± 3.22 per ct. greater than that of the plants which adjoined leaf roll plants. From this it appears that leaf roll plants, even tho small, do not have the effect of missing hills.

In this connection it may be noted that, in marketable tubers, the average yield of the leaf roll plants was less than one-third that of the normal plants. Altho the conditions were such as to make close comparison impossible, it is plain that leaf roll reduced the yield enormously.

[7] The mean of 26.8 per ct., being about five times as large as its probable error (± 5.42), may be confidently accepted as approximating the truth; but the difference between the two means (4 per ct.) is considerably smaller than the probable error, and therefore may have been due solely to chance.

APPLICATION OF RESULTS.

The statement made in the introduction, that the loss from missing hills probably varies considerably with the variety, the distance between hills, the character and fertility of the soil and the cultural and weather conditions, should be repeated and emphasized. The fact must not be overlooked that the results obtained in this experiment apply only to a single set of conditions, namely, to the variety Sir Walter Raleigh, planted 15 × 36 inches, on heavy clay loam soil of medium fertility, under weather conditions such as prevailed at Geneva in 1918. They are not of general application. However, let us consider how they may be used when the conditions are such as to make them applicable.

Since the effect of a missing hill is to increase the yield of adjoining plants, it follows that a partial stand gives a larger yield than is indicated by the stand. In other words, a stand of 80 per ct. will give more than 80 per ct. of the yield of a full stand. If this larger percentage be designated "stand value", we may say that a stand of 80 per ct. has a stand value of more than 80 per ct.

In plat experiments with potatoes, the yields of plats which differ much in stand cannot be properly compared until the yields have been corrected for full stand. In such cases it is highly important to know how to correct yield for full stand. The formula frequently used, namely, corrected yield $= \dfrac{\text{actual yield}}{\text{stand}}$, is clearly incorrect, because it is based on the assumption that missing hills are a total loss, which is not true. The formula which should be used is, corrected yield $(CY) = \dfrac{\text{actual yield}}{\text{stand value}}$. For the calculation of stand value we may use the formula, $SV = 1 - \dfrac{m - 0.464s}{n}$ in which m is the number of missing hills per acre, s the number of skips [8] per acre and n the number of hills per acre in a full stand.

Let us see how these two formulas may be applied in the solution of the following problem: On a tenth-acre plat of Sir Walter

[8] A skip is a series of consecutive missing hills. It is assumed that when potatoes are planted 15 × 36 inches skips of more than a single hill are a total loss except for 46.4 per ct. of the yield of one hill; that is to say, the adjoining plants on either side make no more use of a large skip than of a small one, and plants in adjoining rows are not influenced. This may not be strictly true.

Raleigh potatoes planted 15 × 36 inches, there were 100 skips with a total of 232 missing hills. The actual yield was 20 bushels. What was the stand value, and what was the yield per acre corrected for full stand?

In the above problem $m = 232 \times 10 = 2320$; $s = 100 \times 10 = 1000$; and $n = 11600$. Substituting these values in the formula for stand value we have $SV = 1 - \frac{2320 - 464}{11600} = 1 - \frac{1856}{11600} = 0.84$ or 84 per ct. Hence, $CY = \frac{200}{0.84} = 238.1$ and we have the answer: the stand value was 84 per ct. and the yield corrected to full stand was at the rate of 238 bushels per acre.

From the above discussion it will be seen that it is impossible to correct the yield for full stand when only the number of missing hills is known. It is necessary to know both the number of missing hills and the number of skips.

It is to be hoped that other experiments of a similar kind may be made for the purpose of deriving stand-value formulas for other sets of conditions. While it is probably impossible to derive a formula having general application, it seems to the writer quite possible to obtain formulas which will give results approximately correct when applied to particular sets or combinations of conditions.

NOTES ON NEW YORK PLANT DISEASES, II.*

F. C. STEWART.

SUMMARY.

This bulletin is the second number of a series containing brief notes on various plant diseases in New York. The principal items are as follows: The downy mildew of alfalfa occurs almost every season, but rarely causes appreciable damage. The yellow leaf blotch of alfalfa has been found at Geneva. A fruit rot of apples caused by *Leptosphaeria coniothyrium* was observed for the first time on apples from Waterport. Apples apparently perfect at harvest time may show much fruit-pit after two months in common storage. A stem-constriction disease of unknown cause occasionally occurs on apple trees in nurseries. Apple and pear seedlings are much subject to a disease the chief symptom of which is a blackening of the roots and stem at the surface of the soil. The cause has not been determined. The petiole rot of the spotted arum, caused by *Bacillus carotovorus,* is described in detail. A case of proliferation in the inflorescence of *Bromus inermis* is noted. Butternut trees often cast their leaves prematurely in consequence of the attack of an anthracnose fungus, *Gnomonia leptostyla;* but *Microstroma juglandis* is of rare occurrence on butternut leaves. The blackleg disease of cabbage caused by *Phoma lingam,* was first observed on Long Island in 1903. It has since become common and troublesome in some parts of the State. On Long Island, the decaying leaves of cabbage plants designed for seed production and stored during winter in trenches are often thickly covered in spring with shiny, round, brown or black bodies of the size of mustard seed. These are the sclerotia of an unidentified fungus which appears to be responsible

* Reprint of Bulletin No. 463, June, 1919. Notes on New York plant diseases I, the first number of this series, was published in December, 1910, as Bulletin No. 328

for a part of the decay. A disease of cabbage called black leaf-speck is described. A destructive storage rot of carrots is caused by the fungus *Sclerotinia libertiana*. Soft rot, caused by *Bacillus carotovorus*, is often associated with it. The leaf spot and twig blight of catalpa caused by the catalpa midge, *Cecidomyia catalpæ*, is often mistaken for a fungus disease. The leaf spot of cherries, a destructive fungus disease, can be controlled by proper spraying; but the yellow leaf, distinguished by the absence of brown spots, is a non-parasitic malady uncontrollable by spraying. The western sand cherry, *Prunus besseyi*, is very susceptible to twig blight caused by *Sclerotinia cinerea*. *Fomes applanatus* occasionally attacks the trunks of living cherry trees causing heart rot. *Gloeosporium caulivorum*, an anthracnose fungus with sickle-shaped spores, occurs frequently on red clover and sometimes causes considerable damage. *Pseudopeziza trifolii*, which causes a leaf spot disease of red clover, is common but not often destructive. Two rare clover diseases, a Rhizoctonia leaf rot of white clover and a Cereospora leaf blight affecting white and alsike clovers are described. A case of damping-off of field-grown cucumber seedlings was found to be due to the common damping-off fungus, *Pythium debaryanum*. Several diseases of the currant are discussed, viz.: the relative importance of anthracnose and leaf spot; currant berries attacked by *Pseudopeziza ribis;* a Botrytis leaf spot; the angular leaf spot, a rare disease; crinkle leaf, a new disease which is interesting but unimportant; Fomes root rot, in which the causal fungus, *Fomes ribis*, tho plainly parasitic, injures its host but slightly; fruit drop, a widespread trouble due to some sudden check in growth; a root rot caused by *Hypholoma perplexum;* powdery mildew on red and black currants; sunburn of leaves and fruit; tipburn affecting the leaves; failure of buds to develop; a white deposit on the canes; witches' brooms; and yellow leaf, a non-parasitic trouble. Under elm, four diseases are described, viz.: Anthracnose, a leaf disease caused by *Mycosphaerella ulmi;* a non-parasitic yellow-leaf disease; trunk injury induced by a box "protector;" and an interesting case of a tree in which many branches died from some cause which could not be determined.

ALFALFA.

Medicago sativa.

DOWNY MILDEW, *Peronospora trifoliorum* DeBy. This fungus continues to occur frequently in New York alfalfa fields but rarely causes appreciable damage. It may be found from about the middle of May until frost. The earliest date of its appearance noted during the past ten years is May 17, in 1911, and May 18, in 1912. In both of these cases it was found at Geneva on alfalfa plants 10 to 12 inches high. On May 28, 1910, it was abundant at Riverhead, Long Island.

ASCOCHYTA LEAF SPOT, *Ascochyta imperfecta* Pk. Since the publication of Bulletin 305, in which this disease was first described, specimens of affected leaves were sent to the late Dr. C. H. Peck who has described the causal fungus and given it the name *Ascochyta imperfecta* (33).

YELLOW LEAF BLOTCH, *Pyrenopeziza medicaginis* Fckl. This is an addition to the list of alfalfa diseases in New York. It has been fully described by F. R. Jones (18) who has identified the pycnidial form of the causal fungus (the form in which it is found on living leaves) as *Phyllosticta medicaginis* Fckl. and proven the ascigerous form to be *Pyrenopeziza medicaginis* Fckl. Our attention was directed to it by Prof. J. E. Howitt who sent specimens from Guelph, Canada. Upon making an examination of alfalfa fields in the vicinity of Geneva in June, 1912, a little of the disease was found; but further observations have not been made and we do not know how common it may be in the State.

APPLE.

Malus sylvestris.

CONIOTHYRIUM FRUIT-ROT, *Leptosphaeria coniothyrium* (Fckl.) Sacc. In July, 1917, a package containing five green (unripe) apples was received from Waterport, N. Y. Each apple bore one or two sunken brown spots, 3 to 6 mm. in diameter, with conspicuous red borders. The surface of every spot was thickly covered with pycnidia of a species of Coniothyrium, apparently *C. fuckelii*, the pycnidial form of *Leptosphaeria coniothyrium.* This fungus causes a form of canker which is not uncommon on apple wood in New

York (29), but it has not previously come to our attention in the rôle of a parasite on apple fruit. However, it has been reported by Stevens and Hall (38) as causing a fruit rot of apples in North Carolina.

STEM CONSTRICTION. Cause undetermined. Two cases of this have come to our attention. Both occurred in nurseries at Geneva. On July 17, 1901, it was observed that the leaves on several seedling apple trees 14 to 18 inches high were dead. Plainly, this was due to strangulation. On the trunk at the surface of the soil the bark was dead, brown and shrunken all the way round, forming a well-marked constriction about an inch long. Both above and below the constriction the bark was still green, but all leaves above the constriction were dead. On most of the affected plants new shoots had started below the constriction. No fungus was visible on the surface, but microscopic examination revealed the presence of slender, hyalin fungus hyphae in the diseased bark and wood. The fungus was not identified.

On June 20, 1911, Mr. Parrott, the Station Entomologist, brought us a number of seedling apple trees which were wilting in consequence of a constriction of the stem at the surface of the soil. The bark was dead and shrunken tight to the wood over a section about one-half inch long. Apparently, this was an earlier stage of the disease observed in 1901.

In both of the above cases the diseased trees were widely scattered among healthy ones. Perhaps this is related to the stem-and-root disease of apple seedlings described on page 161. Possibly, it is due to excessive heat like the disease of pine seedlings described by Hartley (14). Altho usually fatal the constriction disease is probably of little or no economic importance owing to its rare occurrence.

FRUIT-PIT. Non-parasitic. About October 25, 1913, a quantity of each of 120 varieties of apples grown on the Station farm was picked and put into storage. Only perfect specimens were selected because it was intended to use these apples in the fruit exhibits to be made by the Station during the following winter. During the first three weeks of storage the temperature of the storage room was kept below 50° F. by the use of ice. After about November 15 no artificial methods of cooling or heating were employed.

On December 23 each variety was carefully examined for the purpose of selecting specimens for a fruit exhibit at Rochester. It was then discovered that about 50 per ct. of the apples of one variety, Lehigh Greening, were severely affected with fruit-pit[3] while none of the other 119 varieties grown and stored under parallel conditions were affected. This indicates that the Lehigh Greening is very susceptible to fruit-pit. It appears to show, also, that fruit-pit may develop in storage.

However, the application of the iodin test revealed the presence of considerable starch in the dead, brown tissue underneath the surface spots. This means either that the disease was present at harvest time and overlooked, because the spots did not show on the surface, or else the apples were not fully ripe when picked.

STEM-AND-ROOT DISEASE OF SEEDLINGS. Cause undetermined. During the past ten years the Horticultural Department of the Station has had much trouble with a disease affecting the stem and roots of apple and pear seedlings grown in " flats " in the greenhouse. Usually, the disease has made its appearance early in April when the plants were about one inch high with two to four leaves besides the seed leaves. The browning or blackening (in the case of the pear) of the leaves is the first symptom noticed. Upon examination, the roots and portion of the stem below the surface of the soil are found dead and black. Frequently, plants are found with a short section of the stem at the surface of the soil dead and shriveled while parts above and below this point appear normal. This appears to be the early stage. Many plants so affected recover without ever showing any leaf symptoms. In fact, many plants showing pronounced root and leaf symptoms ultimately recover. The mortality among affected plants is not as high as one might expect considering the condition of the roots.

The symptoms of the disease suggest a parasitic organism as the cause. On several occasions the writer has made a microscopic examination of the roots and stems of affected plants without finding any fungus which might be held responsible for the trouble. Neither is there any ocular evidence of the presence of bacteria in unusual numbers. Nevertheless, in the season of 1911, when the soil used was partially sterilized, there were fewer diseased plants than usual.

[3] The disease described by the author in Bul. 164 of this Station, pp. 215–219, and by Brooks in *Bul. Torrey Bot. Club*, 35: 430–432.

Suspicion having fallen on the fertilizer used, the nitrate of soda in particular, chemical fertilizers were entirely omitted in 1909 without the least diminution in the amount of disease.

ARUM, SPOTTED.

Amorphophallus simlense.

PETIOLE ROT, *Bacillus carotovorus* Jones. In an article by H. A. Harding and the present writer (12), published in *Science* about sixteen years ago, mention was made of a bacterial soft rot of the petioles of *Amorphophallus simlense.* At the time, it was the intention of the authors to publish a more complete account of the disease later. This idea being now abandoned, it seems desirable to publish a note on the subject here.

Amorphophallus simlense is one of the giant aroids grown for ornament or as a curiosity. In the open, it flowers in early spring, then throws up a single large leaf with a spotted, succulent petiole one to two feet in length and three-fourths of an inch to one inch in diameter. This leaf, if not attacked by disease, persists until frost.

The disease in question first came to the writer's attention at Floral Park, Long Island, early in June, 1897. At this date it was already scattered all thru a plat of about 4000 plants in three rows about twenty rods long. As the season advanced the plants one by one succumbed to the disease until by September 1 about 25 per ct. were dead. During the next five years the disease was present at the same place each year, but less destructive than in 1897. No observations have been made since 1902.

The disease first attacks the petiole at or below the surface of the soil. The affected tissue may be either moist and water-soaked in appearance or dry and brown. In either case the discoloration extends rapidly from the base of the petiole to its summit. In plants attacked during the early part of the season the diseased tissue is very moist, soft and foul smelling and the epidermis may be easily removed by rubbing with the fingers. In plants attacked after the middle of July the diseased tissue is usually brown and quite dry. Whether the rot assumes the wet or the dry form appears to depend more upon the age of the leaf than upon the state of the weather.

Almost from the first appearance of the disease the leaf blade begins to change color and by the time the rot has reached the summit of the petiole the blade is yellow. The whole leaf now falls to the ground and rapidly decays. A prominent character of the disease is its tendency to run up one side of the petiole while the opposite side remains sound. It is very common to find petioles with a wide strip on one side rotten from bottom to top while the opposite side is sound thruout the entire length. When the affected leaf topples over it usually falls with the diseased side down.

The disease is remarkably rapid in its action. Frequently the attacked leaf falls within four days after the first symptoms of disease. Among eleven leaves artificially inoculated during the latter part of June three fell within 100 hours after inoculation.

No new leaves are put forth to replace the dead ones. The tubers do not rot, but it appears probable that they serve as carriers of the disease.

As stated in the article above mentioned, inoculation experiments with pure cultures have shown *Bacillus carotovorus* to be the cause of the disease.

BROME GRASS.

Bromus inermis.

PROLIFERATION OF THE INFLORESCENCE. On July 11, 1915, the writer's attention was attracted by the peculiar appearance of some brome grass growing by the roadside near Geneva. Many of the heads had a leafy appearance. Upon examination it was found that the leafy appearance was due to the transformation of some or all of the spikelets into leafy shoots. The length of the leafy shoots varied, but, usually, it was two or three times that of a normal spikelet.[3]

BUTTERNUT.

Juglans cinerea.

ANTHRACNOSE, *Gnomonia leptostyla* (Fr.) Ces. & De Not. Butternut trees often cast their leaves prematurely in consequence of the attacks of a fungus thought to be identical with that causing the anthracnose of black walnut leaves. Affected leaves show irregular brown spots of all sizes from a mere speck up to about

[3] Host Herbarium Specimen No. 75.

three-eighths of an inch across. On the tips and margins of the leaves the spots frequently coalesce and form large, dead, brown areas. Acervuli of the conidial stage of the causal fungus (known as *Marssonia juglandis* (Lib.) Sacc.[4] occur on both surfaces of the spots. Hyalin, two-celled, sickle-shaped conidia are abundant. Usually, September is the season of the most conspicuous effects of the disease; but, according to Duggar (9), " almost complete defoliation of some trees has been noted as early as the latter part of July in New York."

WHITE MOLD, *Microstroma juglandis* (Bereng.) Sacc. This, also, is a fungus disease affecting butternut leaves but it is rare and unimportant in New York. It is recognized by the small patches of white mold which appear on the under surfaces of the leaves. In specimens collected by the writer at Milton, N. Y., September 2, 1901, the causal fungus had spores like those described and illustrated by Peck (31, p. 30 and pl. I, figs. 15–17) for a fungus which he found on butternut leaves at Charlton, N. Y.[5] Peck referred his fungus, doubtfully, to *Microstroma leucosporum* Niessl; but Saccardo (34) gives it as *M. juglandis* var. *brachysporum*.

CABBAGE.

Brassica oleracea.

BLACKLEG. *Phoma lingam* (Tode) Desm. Apparently, this troublesome disease of cabbage is becoming quite prevalent in some parts of New York, particularly in Ontario County where it has been reported repeatedly as causing serious damage during the past three or four years. The variety most frequently affected is Allhead Early. In some cases the diseased plants are scattered irregularly thru the field, but it often happens that many plants in one row are dead or dying with the disease while the plants in the adjoining row are all healthy. This leads to the belief that the disease is frequently contracted in the seedbed and disseminated with the seedlings. It has been proven by Henderson (16) that the disease may originate in infected seed. Also, it appears probable that a diseased seedling may infect others with which it comes in contact in the process of transplanting. The common practise

[4] *Gloeosporium juglandis* (Lib.) Mont. is a synonym.
[5] Host Herbarium Specimen No. 76.

of bunching the seedlings and placing the roots in water preparatory to transplanting is highly favorable to such infection. This may account for the marked tendency of the disease to appear in certain rows and not in others. Probably the disease may also be spread from one plant to another in the same row by cultivation.

The danger arising from the use of infected seed may be largely, but not wholly, avoided by soaking the seed for fifteen minutes in a 1-1000 solution of corrosive sublimate as recommended by Harding *et al* (13) for the prevention of blackrot, or by "immersion for twenty to twenty-five minutes in a 1:200 solution of 40 per ct. formaldehyde, followed by washing in clear water," as recommended by Henderson (16, p. 430).

How long the blackleg disease has been in America is unknown. It was first definitely recognized by Manns (26) in Ohio in 1910, but its occurrence in Minnesota as early as 1906 is indicated by a statement made by Washburn (44), State Entomologist of Minnesota, in his report of that year. It is quite possible that it may have existed here several years but been confused with other root troubles.

In this connection it is worthy of note that the writer has positive knowledge of the occurrence of cabbage blackleg on Long Island in 1903. It was at Riverhead in a field of one and one-half acres of cabbage which had been planted late for the purpose of raising plants to set out for seed production the following season. On November 6, when our observations were made, between 35 and 50 per ct. of the plants were either dead or dying. Most of the dead plants had succumbed quite recently and were not yet dry. They were scattered irregularly over the field. Usually, the roots of affected plants had died and decayed leaving only the stem which, also, was frequently badly decayed in its lower portion. In some cases the roots were in fair condition, but the plant was rendered worthless by a large canker on the stem. In the woody portion of the lower part of the stem there were black streaks resembling the blackened bundles found in the stems of plants attacked by blackrot, but this was evidently not a case of blackrot because the black streaks did not extend up the stem as far as the attachment of the leaves and scarcely any of the plants showed leaf symptoms of blackrot. Some plants showed evidence of injury by cabbage maggots, but the bulk of the trouble was plainly due to some

other cause. Rhizoctonia, also, was readily eliminated as a possible cause. Finally, it was observed that the stems of affected plants almost invariably bore pycnidia of a species of Phoma. These pycnidia were not readily detected with the unaided eye, but by washing the stems and making use of a hand lens it was easy to find Phoma pycnidia on the stem of practically every affected plant. Every one of 32 affected plants selected at random showed the pycnidia. The conclusion was reached that the Phoma found on the stems was the probable cause of the trouble, but the identity of the fungus remained unknown until the appearance, in 1906, of an article by J. Ritzema Bos (4) describing a similar disease of cabbage in Holland caused by *Phoma oleracea* Sacc. (=*Phoma lingam* [Tode] Desm.).

STORAGE ROT. Fungus undet. On Long Island, cabbage plants intended for seed production are stored over winter in shallow trenches made with a plow. The plants are set in the trench close together, heads up, and covered with dirt by throwing a couple of furrows over them from both sides. The following spring, about April 1, the dirt covering is removed and such plants as are still sound are set out to produce seed. There is always some, and often considerable, loss from rot which is partly due to freezing, partly to the attacks of the soft rot organism, *Bacillus carotovorus* Jones, and partly to a fungus which produces large numbers of sclerotia.

This note has to do with the sclerotia. They are invariably present when the trenches are opened and often occur in such large numbers as to attract attention. Some of hem are to be found on almost every decaying cabbage leaf. In different stages of maturity they are white, yellowish, reddish-brown and black. When mature they are usually the size of a large mustard seed (sometimes considerably larger) roundish, black, and shiny, and but slightly attached to the leaf.[6] (Plate II, fig. 1.) As a rule, very little mycelium is in evidence and no fruiting stage has been found in association with the sclerotia. Attempts to germinate the sclerotia have failed.

In this connection there should be mentioned some sclerotia found on decaying cabbages stored in a cellar at the Experiment

[6] Museum Specimens Nos. 142 and 143.

Station. These were roundish, black, and shiny and closely resembled the sclerotia found on Long Island except that they were somewhat smaller and associated with a luxuriant sterile growth of coarse, white mold. (Plate III, fig 1.) Under the microscope the white mold was found to consist of branched, tree-like structures composed of bundles of hyphae packed together as in the stipe of an agaric. Often these tree-like structures sprung directly from sclerotia. (Plate III, fig. 2.)

While it is unknown to what fungus either of these two kinds of sclerotia belong, it may be said that they do not belong to *Sclerotinia libertiana* which is sometimes parasitic on cabbage plants (15). Some sclerotia of *Sclerotinia libertiana* are shown in Plate II, fig. 2, for comparison. These were taken from a mother seed plant which was wilting and dying from an attack of a cottony, white fungus (*Sclerotinia libertiana*) on the lower portion of the stem.

BLACK LEAF-SPECK. Cause undetermined. In April, 1911, a cabbage grower of North Tonawanda sent to the Station some specimens of diseased cabbage leaves accompanied by the following letter: "I send you some cabbage leaves of the Danish [Danish Ballhead] variety, and would like to have you tell me what it is that affects them. This is only an ordinary sample, some being much worse. There was quite a large proportion of the crop affected. It was unsalable. It does not show until January when the cabbage gets white. We store in the barn where cabbage is kept just above freezing point. We first noticed it a year ago on the crop of 1909. Can you give a remedy?"

The specimens sent were white leaves from the interior of a cabbage head. They were plentifully sprinkled with minute black spots varying in size from a mere point to the size of a small pinhead. Many of them resembled fly specks. Most of the spots were broadly elliptical, their major axes lying parallel with the larger veins of the leaf. In location, they bore no definite relation to the veins. Some were seated on the veins, while others were on the parenchyma between the veins. Some were visible on both sides of the leaf, others on one side only. Sometimes the spots occurred singly, and sometimes in irregular groups. (Plate IV, fig. 2.) With the aid of a hand lens it could be seen that the surface of the spots was slightly sunken.

What appears to have been the same disease was observed at Canandaigua in the fall of 1910. In this case, the plants became affected while still in the field, and the outer as well as the inner leaves showed the spotting. In specimens of affected leaves received from Long Island in February, 1915, the spots, tho typical in other respects, were visible only from one side of the leaf.

The writer has been unable to determine the cause of this disease or connect it, definitely, with any of the described diseases of cabbage. However, there is a possibility that it is McCulloch's bacterial spot disease caused by *Bacterium maculicolum* (25).

The apparent absence of fungus hyphae from the diseased tissue (as demonstrated by microscopic examination of free-hand sections) indicates that no fungus is responsible for it.

CARROT.

Daucus carota.

STORAGE ROT, *Sclerotinia libertiana* Fckl. and *Bacillus carotovorus* Jones. Where carrots are stored in large quantities, either in cellar storage or cold storage, one of the regular troubles is a form of rot in which areas of softened tissue are covered with a copious growth of white mold. Several times during the past twenty years carrot growers on eastern Long Island have complained to the Station of heavy losses due to this rot. Cold storage houses in Rochester, also, have occasionally reported loss from the same cause.

The white mold is *Sclerotinia libertiana* Fckl. It may be identified by the large, black, somewhat irregular sclerotia which are produced in considerable numbers on the decaying carrots. The writer's studies have convinced him that this fungus alone may cause extensive decay of stored carrots; but, usually, there is associated with it an active soft rot bacillus, probably *Bacillus carotovorus* Jones, which hastens the decay and greatly increases the amount of damage done. *Botrytis vulgaris* and species of Fusarium and Pencillium, also, are frequently associated with the decay.

The idea that *Sclerotinia libertiana* may cause a rot of carrots is not new. The literature of plant diseases contains numerous references to it. Stevens (37) has described a form of carrot rot caused by a fungus which produces sclerotia resembling mustard seeds.

CATALPA.

Catalpa spp.

TWIG BLIGHT AND LEAF SPOT, *Cecidomyia catalpae* Comstock. From time to time the Botanical Department of the Station has been called upon to explain the nature of a twig blight and leaf spot of young catalpa trees the symptoms of which strongly suggest parasitic fungi or bacteria as the cause. The writer has been much puzzled by these troubles, but has reached the conclusion that the twig blight and a part, at least, of the leaf spot are caused by an insect, the catalpa midge, *Cecidomyia catalpae* Comstock.

The twig blight and leaf spot may occur in association or separately. In August and September short shoots of the current season's growth are found to be dead, blackened and shriveled. Upon cutting open the dead shoots one may or may not find small, yellowish larvæ of the catalpa midge. How early the larvæ may occur the writer does not know, but he has found them abundant the latter part of September. The spots on the leaves are brown, circular and commonly 3 to 7 mm. in diameter. They may be either destitute of fungi or bear numerous pycnidia of a species of Phyllosticta (*P. catalpæ* E. & M.). Whether the Phyllosticta is always responsible for the spots on which it occurs is not known; but there is good reason to suspect that spots which do not bear fungi are the work of the catalpa midge.

A full account of the catalpa midge and its work are given by Gossard in Bulletin No. 197 of the Ohio Experiment Station.

CHERRY.

Prunus spp.

LEAF SPOT, *Coccomyces hiemalis* Higgins. This is a common fungus disease in which the leaves first show numerous small brown spots, then turn yellow and fall prematurely. In the seasons of 1916 and 1917, owing to long continued spells of wet weather in the spring, leaf spot was unusually prevalent and destructive over the greater part of the State. In many orchards of sour cherries the trees were almost completely defoliated before the fruit was fully ripe. Many trees were so much weakened that they afterward died of winter injury.

Fruit growers who have attempted to control the disease by spraying have sometimes succeeded and sometimes failed to a greater or less extent. As is often the case, it is uncertain whether the failures reported were due to the method of treatment being inherently faulty or to its having been improperly or carelessly applied. Failure to obtain good results from spraying is often due to lack of thoroness.

In a recent report on experiments made in Wisconsin, Keitt (20) recommends plowing under the fallen leaves before the blossoms open and spraying two or three times with bordeaux mixture, 3–3–50, or lime-sulfur, 1–40. The first spraying should be made soon after the petals fall and a second one two weeks later. If a third spraying seems necessary it should be made as soon as the fruit is harvested. In each application add arsenate of lead to the fungicide at the rate of three-fourths to one pound of the powder or one and a half to two pounds of the paste to each fifty gallons.

YELLOW LEAF. Non-parasitic. Occasionally the leaves of sour cherry trees turn yellow and fall prematurely in large numbers without being attacked by any fungus or insect. This trouble is readily distinguished from leaf spot by the absence of brown spots on the leaves. It is caused by unfavorable soil and weather conditions and is not preventable by spraying.

WINTER INJURY (?). Cherry trees often die unexpectedly and mysteriously. In many cases death occurs quite suddenly after the leaves appear in spring. Sweet cherries, particularly, are liable to go this way. An examination of all parts of the tree, including the roots, usually fails to reveal the cause of the trouble and death is vaguely attributed to "winter injury." While this diagnosis may be correct, in a general way, it is highly desirable to know more definitely how the death of the trees is brought about and what conditions induce it.

A puzzling case which may, perhaps, be classed with winter injury troubles came under the observation of the writer recently. It occurred at Geneva in a cherry orchard about twenty years old. The variety was Montmorency. Thruout the season of 1917 seventeen trees showed symptoms of ill health.

Up to the spring of 1917 it had not been observed that any of the trees were abnormal. The orchard contained a full stand of fairly thrifty trees quite uniform in size and appearance. The

affected trees all stood in one row tho not in consecutive order. This fact aroused the suspicion that the trees in this row had received different treatment from those in the remainder of the orchard, but diligent inquiry failed to discover confirmatory evidence. However, it is possible that this row failed to receive an application of nitrate of soda given the remainder of the orchard in the summer of 1916 to counteract the effect of a severe attack of leaf spot.

There seemed to be no difference in soil conditions which would account for the trouble. The surface drainage and underdrainage were both good and the same where the affected trees stood as elsewhere. Around one of the affected trees the soil was removed over a radius of about thirty inches and to a depth of three feet. All of the roots thus exposed were alive and apparently normal.

The blasting of the blossoms was the first symptom to attract attention. Soon after blooming the affected trees became conspicuous by the small size of their leaves. By June 8, 1917, it appeared that the trees would surely die, but all lived thru the summer.

As an experiment, six of the trees were severely pruned ("dehorned"). This proved beneficial. The pruned trees produced many new shoots twelve to thirty inches long having large leaves. The unpruned trees (with two exceptions) made no new growth whatever while normal trees added from two to six inches of new growth at the tips of their branches. The leaves of unpruned affected trees were fewer, smaller and darker green than the leaves of normal trees.

In the spring of 1918, after an unusually severe winter, the affected trees were all still alive. By June 5 the "dehorned" trees had made new shoots six to ten inches long, but bore no fruit. The unpruned trees were very scant of foliage and had made no new wood growth, but they bore a little fruit. It now appears that less severe pruning might have been more effective in rejuvenating the affected trees.

TWIG BLIGHT, *Sclerotinia cinerea* (Bon.) Schröt. On June 11, 1918, a block of the western sand cherry, *Prunus besseyi*, in a Geneva nursery was found to be severely affected with twig blight caused by the brown-rot fungus. The affected plants were bushes consisting of twelve to twenty-five willow-like shoots three to four feet high. Originally, they were dwarf peach trees on sand cherry stocks; but the peach trees, having made an unsatisfactory growth, were cut

back to within a few inches of the ground in the hope that new and better tops would be produced from dormant buds. For some reason the peach buds failed to develop and sprouts from the stocks came up instead.

About 25 per ct. of the shoots were blighted at the top, the length of the blighted portion varying from six to eighteen inches. While some of the shoots had died quite recently, others had been dead for some time. The causal fungus had first attacked and blighted the blossoms, then passed down the flower pedicels into the axis of the shoot where the bark and wood were killed and discolored over a section one-half to one and one-half inches in length. Usually, the point of attack was indicated by a drop of exuded gum; and gray tufts of Monilia conidia were often to be seen on the bases of small, lateral shoots nearby. Occasional fruits attacked and killed by the fungus also bore tufts of conidia. The leaves on all parts above the stem lesion wilted and died from lack of water.

This case is of interest because the host plant, *Prunus besseyi*, is one of the parents of the hybrid Compass plum previously reported by the writer as being severely attacked by the Monilia stage of *Sclerotinia fructigena* in a similar manner (40). Altho the causal fungus is probably the same, the name used here is different. Recent studies by Matheny (28), Bartram (2) and others indicate that the fungus causing brown-rot of stone fruits in this country is referable to *Sclerotinia cinerea* rather than to *S. fructigena.*

TRUNK ROT, *Fromes applanatus* (Pers.) Wallr. The writer has twice observed sporophores of *Fomes applanatus* on the trunks of living cherry trees.

In one case (at Shortsville) the affected tree belonged to a small-fruited variety of sweet cherry (*Prunus avium*). Its trunk was about two feet in diameter and, except for a narrow crack, appeared sound externally tho undoubtedly somewhat affected by heart-rot within. At the top, the tree was alive with the exception of one large branch which was dead. A single well-developed sporophore of *F. applanatus* about six inches wide sprung from the crack in the trunk about two feet above the ground.

The other case (at Geneva) also involved a sweet cherry tree of unknown variety. The trunk was about a foot in diameter. Its condition is not known except that the wood was nowhere exposed. It bore a sporophore of *F. applanatus* at a height of about ten inches

above the ground. Dead branches scattered thru the top indicated that the vigor of the tree was decidedly on the decline.

CLOVER.

Trifolium spp.

ANTHRACNOSE, *Gloeosporium caulivorum* Kirch. Early in June, 1914, the writer was asked to investigate the unhealthy condition of some red clover fields in the vicinity of Seneca Castle. In one field of several acres the crop of hay was nearly ruined. The clover stems were still green, but most of the leaves were dead and black and many had fallen. Two other fields on the same farm were injured in a similar manner but less severely. Severe attacks of the same disease occurred also at Geneva, Newark and South Lima. The trouble was due to an anthracnose caused by the fungus *Gloeosporium caulivorum* Kirch.[7]

As no observations were made in 1915 or 1916, nothing is known about the occurrence of the disease in those years.

In 1917 it was first observed at Geneva on June 6 in a field in which, at this time, the clover stood twelve to fourteen inches high but had not yet commenced to bloom. To the casual observer the crop in this field appeared to be in excellent condition; but close examination revealed the fact that it was thoroly infected with anthracnose thruout. Here and there leaves were hanging limp beside their stems because their petioles were soft rotten (Plate I). While some of the hanging leaves were dead and black, the majority were still green. This indicated that they had been attacked recently. The weather being showery they had not shriveled as quickly as they would have done had it been dry. Usually, there was only one affected leaf on a stem, namely, the third one from the top. Occasionally, the fungus had attacked one of the large veins in the leaf causing a V-shaped, dead, brown area. Spores of the causal fungus were plentiful on the lesions. Lesions on the stems were rare at this time, but became quite numerous later. Notwithstanding the infection of the plants was general and the weather almost continuously wet, it was about two weeks before the ravages of the disease became conspicuous. A fair crop of hay was harvested.

[7] Host Herbarium Specimen No. 77.

More destructive outbreaks were reported from Seneca County and McConnellsville.

Gloeosporium caulivorum, the causal fungus, was first described and illustrated in 1902 by Kirchner (21) who studied an outbreak of the disease in Germany. According to Stevens and Hall (39) it was "first reported in the United States by Sheldon (35) in 1906 and has since been noted as serious in a number of states." However, its American literature is very meager, the most important paper being that of Fulton (11) published in *Science* in 1910.

As usually found, the stems and leaf petioles of diseased plants show black streaks or elongated, black, sunken spots covered with acervuli of the causal fungus. Identification of the fungus is made easy by the fact that its spores are strongly curved, while those of *Gloeosporium trifolii* Pk. and *Colletotrichum trifolii* Bain & Essary, the other two anthracnose fungi of clover, are straight.

Undoubtedly, this disease is of more frequent occurrence and greater importance in New York than is indicated by the few cases here recorded. It is not a new disease in this State. The Station herbarium contains specimens collected by the writer at Geneva in 1903.

LEAF SPOT, *Pseudopeziza trifolii* (Pers.) Fckl. A field of red clover near Geneva was quite severely attacked by this disease during the latter part of June, 1914. The upper leaves on almost every plant were affected. Owing to the occurrence of anthracnose (*Gloeosporium caulivorum*) and rust (*Uromyces fallens*) in the same field it was difficult to estimate accurately the amount of damage done by leaf spot. The aggregate loss in yield from the three diseases probably exceeded 25 per ct. of the crop. However, the actual loss must have been greater than this because hay made from plants so much diseased must be of inferior quality.

The spots show, conspicuously, on both surfaces of the leaf. They are brown, roundish or irregular, one to three millimeters across, and often coalesce forming large, dead, brown areas. With the aid of a hand lens apothecia may be seen on some of the spots. At first the apothecia are merely rounded brown elevations, but later they become cup-shaped. They occur on both surfaces of the leaf and contain asci and paraphyses in abundance, but at the time of our latest observations (June 30) ascospores were still rare.[8]

[8] Host Herbarium Specimen No. 78.

Altho clover leaf spot is frequently mentioned in the literature, the accounts of it are nearly all brief. It seems to be generally considered an unimportant disease. No mention of it is made in any of Peck's Reports (30). However, it appears to be rather common, tho probably not often destructive, in New York. It often attacks the lower leaves as well as the upper ones.

The causal fungus is closely related to the fungus of alfalfa leaf spot, *Pseudopeziza medicaginis*. In fact, some mycologists consider it identical with that species, or, at least, merely a form of it. (19, 27)

LEAF BLIGHT, *Cercospora* sp. This is a rare disease. It has come to our attention but once. In this case it was on the leaves of white clover (*Trifolium repens*) at several different points in a Geneva park. Leaves of alsike clover (*T. hybridum*) growing with the white clover were similarly affected. It appeared during wet weather in the latter part of August, 1912.

The affected leaves were covered with irregular, dead, brown spots which varied greatly in size and shape, often being quite large. On the upper surface the spots were thickly dotted with tufts of conidiophores which could be detected with a hand lens. Microscopic examination revealed the presence of typical Cercospora spores varying greatly in size and considerably in the number of septa.[9] It has not been determined to what species of Cercospora the fungus belongs.

Chester (6) uses the name *Cercospora helvola* var. *medicaginis* for a fungus which he found on red and crimson clovers and alfalfa in New Jersey. Ellis and Everhart (10) record *Cercospora helvola* on white clover at Newfield, N. J. Peck (31, p. 29) mentions the occurrence of *Cercospora zebrina* on *Trifolium agrarium* at Sandlake, N. Y. No. 461 of Ellis and Everhart's Fungi Columbiani is a specimen of *Cercospora zebrina* on *Trifolium agrarium* collected at New Brunswick, N. J.

LEAF ROT, *Rhizoctonia* sp. In a luxuriant growth of white clover (*Trifolium repens*) at Geneva, large numbers of the lower leaves became rotten. The affected leaves had a water-soaked appearance. They were loosely bound together and their tissues traversed in all directions by Rhizoctonia hyphæ. Also, sclerotia of Rhizoctonia were attached to petioles of some of the leaves.

[9] Host Herbarium Specimens Nos. 79 and 80.

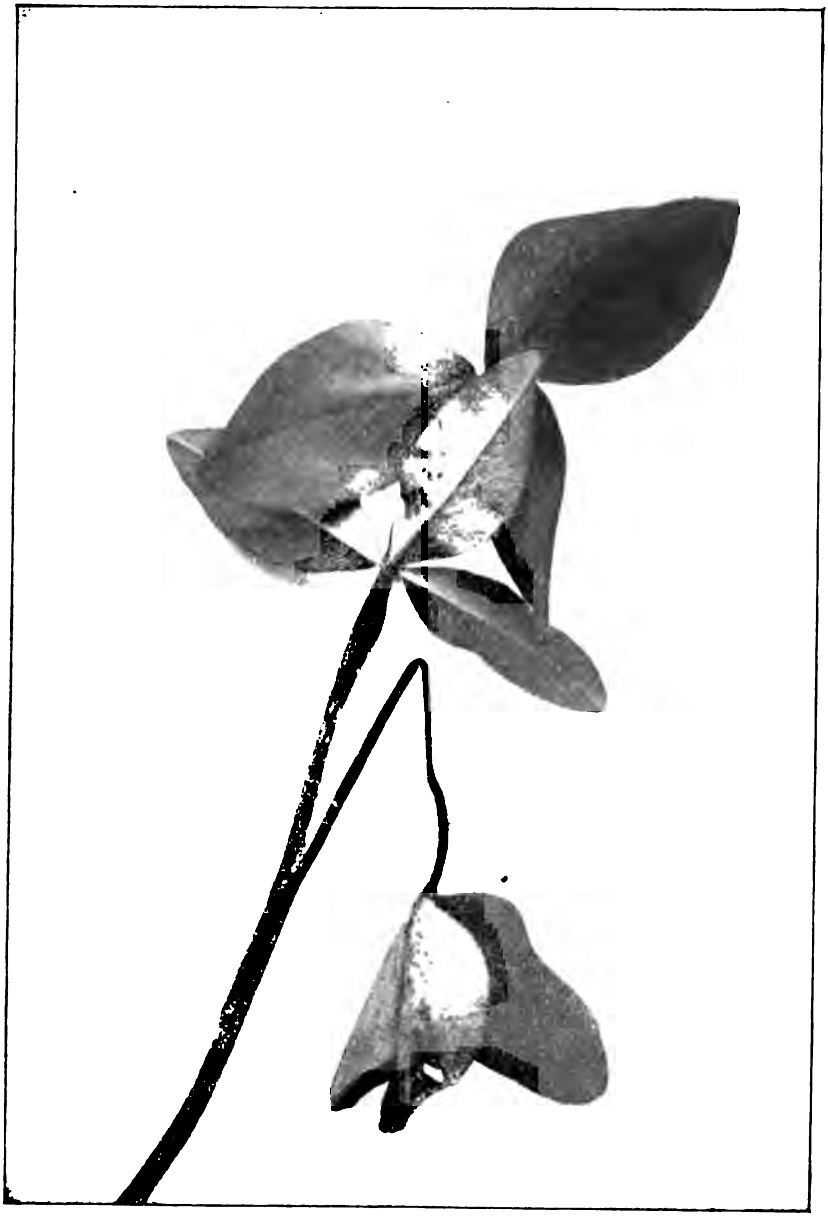

PLATE I.— ANTHRACNOSE OF CLOVER.

Upper portion of a stem of red clover with a leaf whose petiole has been attacked by *Gloeosporium caulivorum*.

(Natural size.)

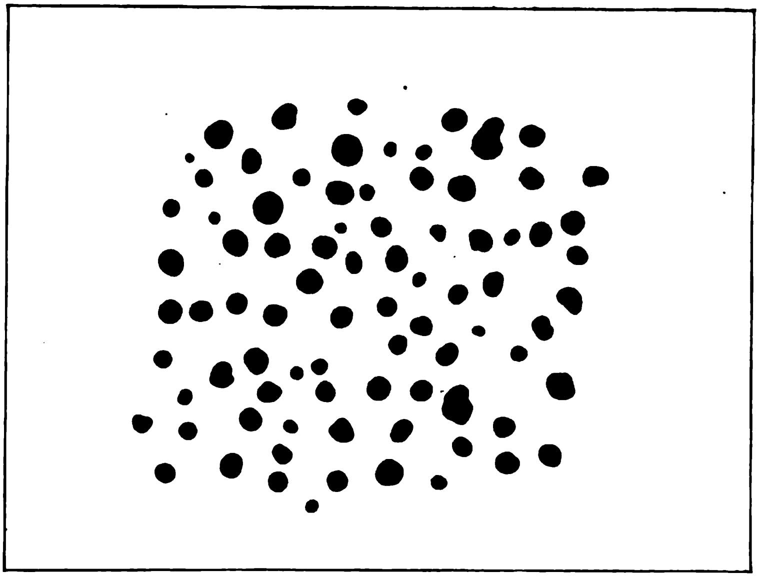

Fig. 1. Sclerotia of unknown fungus; from decaying leaves of cabbage plants stored in trenches.

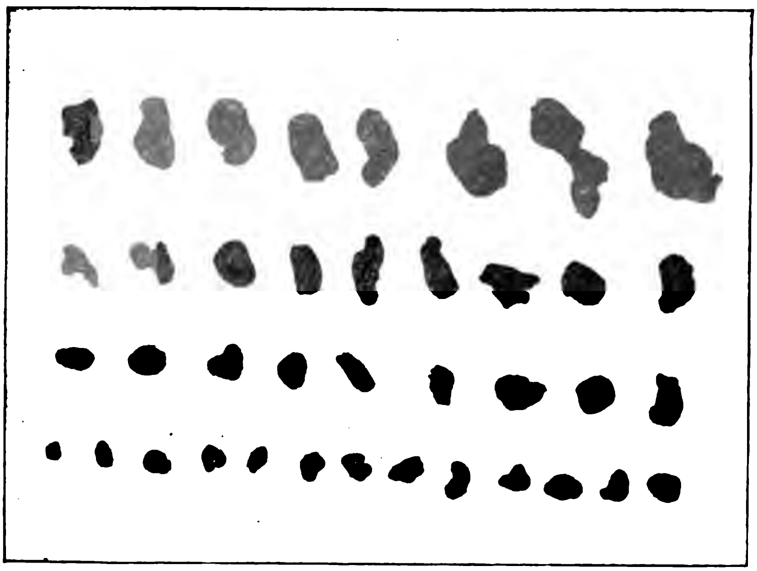

Fig. 2. Sclerotia of *Sclerotinia libertiana;* from a mother seed plant affected with stem rot.

PLATE II.— SCLEROTIA OF CABBAGE ROT FUNGI.

(Both figures natural size.)

Fig. 1. Portion of decaying leaf with growth of white fungus (at left) and black sclerotia (at center).

Fig. 2. A strand of white fungus springing from a sclerotium.

PLATE III.— FUNGUS ON CABBAGE STORED IN A CELLAR.

(Fig. 1 natural size; fig. 2 magnified several times.)

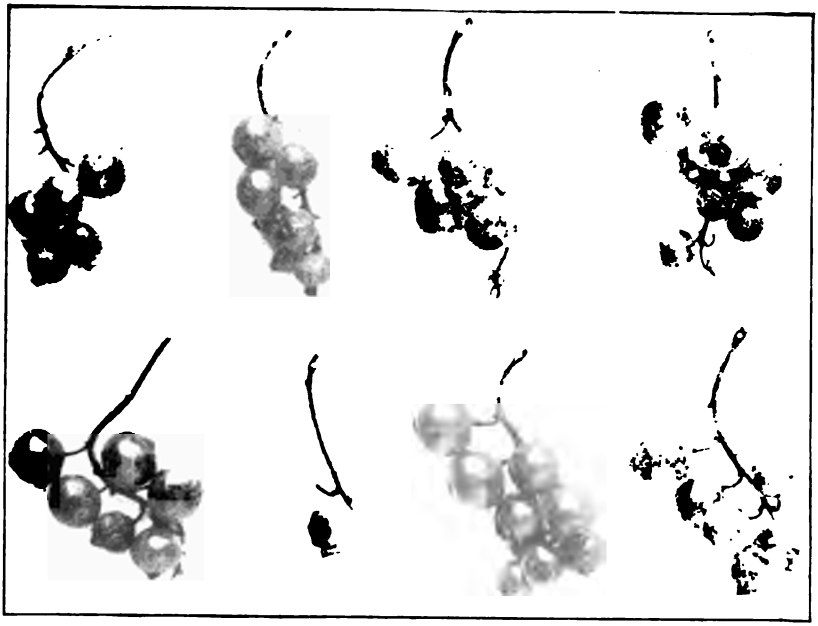

Fig. 1. Berries of red currant attacked by *Pseudopeziza ribis*.

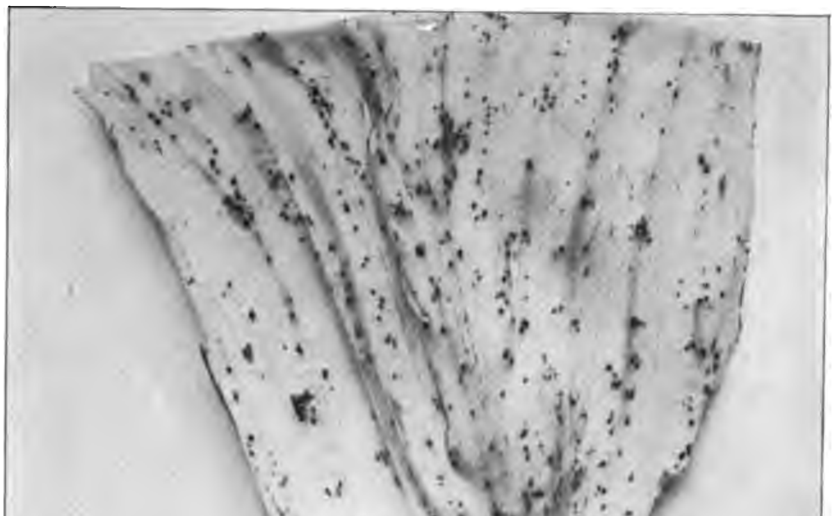

Fig. 2. Portion of cabbage leaf affected with black leaf-speck.

PLATE IV.—ANTHRACNOSE OF CURRANTS AND BLACK LEAF-SPECK OF CABBAGE.

(Both figures natural size.)

Plate V.— Witches' Broom on Red Currant.
(One-half natural size.)

PLATE VI.—*Hypholoma perplexum* ON LIVING CURRANT PLANT.
(Three-fourths natural size.)

Plate VII.—*Fomes ribis* **on Living Currant Plant.**

PLATE VIII.— ELM TREE AFFECTED WITH UNKNOWN DISEASE.

This happened during a period of very wet weather on the latter part of August, 1912. Probably it is a rare occurrence.

CUCUMBER.

Cucumis sativus.

DAMPING OFF, *Pythium debaryanum* Hesse. On July 12, 1917, some specimens of diseased cucumber seedlings were received from Shortsville. They were field-grown plants which had suddenly toppled over soon after the appearance of the seed leaves above ground. The owner stated that a considerable percentage of the plants in a field of about two acres were similarly affected.

Upon seeking an explanation of the trouble it was found that the stems of the seedlings were soft rotten at the surface of the soil and the constant presence of Pythium oogonia in the decaying tissue pointed to *Pythium debaryanum* as the cause.

Pythium debaryanum is notorious as one of the fungi which cause damping-off of seedlings of many kinds in greenhouses and gardens. Its ravages among field crops are less known. In the present instance a period of warm, wet weather furnished conditions favorable for the attack.

FASCIATION. A cucumber plant of the variety Telegraph, growing in the Station greenhouse in the summer of 1917, had a fasciated stem. The plant was about seven feet high. At the surface of the soil the stem was nearly cylindrical and three-fourths of an inch in diameter; but it gradually flattened and widened until it attained a width of 2.5 inches at the top. The fasciated portion bore a single large branch which was entirely normal. It also bore normal flowers and fruit.

CURRANT.

Ribes vulgare and *R. nigrum.*

ANTHRACNOSE, *Pseudopeziza ribis* Kleb. In Bulletin 199 of this Station (41, p. 69) the opinion was expressed that the Septoria leaf spot is the chief cause of the premature dropping of currant leaves in New York. More extended observations made during the past seventeen years lead us to believe that, on the whole, anthracnose is considerably more abundant and important than the Septoria leaf spot. Often, the two diseases work together. The virulence of anthracnose varies greatly from year to year. It may be epidemic

one season and almost wholly absent the next. In the Hudson Valley, where it wrought great havoc in 1901, only traces of it could be found in 1902. It caused almost complete defoliation of currants in the Hudson Valley in the years 1903, 1909 and 1912, and in central New York in 1910 and 1914; while the years 1907 and 1908 were notable for the almost complete absence of the disease from all parts of the State.

Apparently, the damage done by anthracnose and Septoria leaf spot is not as great as one might expect from the extent of the defoliation which they cause. Altho both diseases may be controlled by spraying, either with lime-sulfur solution or with bordeaux mixture, it is doubtful if spraying is profitable on the average.

During a severe outbreak of anthracnose at Kenwood in June, 1910, large numbers of the currant berries were attacked. A few affected berries were observed, also, at Milton in 1909. On the berries the disease takes the form of small, dark brown or black spots resembling fly specks (Plate IV, fig. 1). These spots bear apothecia containing the characteristic curved spores of the anthracnose fungus. The occurrence of *P. ribis* on currant berries has been noted previously by Stewart and Eustace (41, p. 65) and by Clinton (8, p. 12).

The variety Victoria, tho not immune, is notably resistant to anthracnose. Evidence of this has been seen repeatedly. In the latter part of July, 1909, the writer made some observations on anthracnose in a plantation of about 3300 bearing currant bushes at Ripley. The great majority of the bushes were of the variety Fay's Prolific; but bushes of Victoria were scattered here and there all thru the plantation and at one side there were also a few solid rows of this variety. The bushes of Fay's Prolific were so much defoliated by anthracnose that the fruit had been considerably injured by sunscald. The bushes of Victoria, on the contrary, were all in full foliage and almost free from anthracnose. Only a few were slightly affected. The contrast was striking.

BOTRYTIS LEAF SPOT, *Botrytis* sp. At Geneva, in September, 1907, the writer found occasional leaves of red currants showing circular, dead, brown spots one-fourth inch to one inch in diameter. No fungus was visible on the upper surfaces of the spots, but the under surfaces were covered with a growth of conidiophores of Botrytis which was visible to the unaided eye. With the aid of a hand lens the masses of spores on the tips of the conidiophores could be easily seen.

During June, 1909, similar brown spots one-fourth to one-half inch in diameter were plentiful on leaves of red currants at Milton. Sometimes the spots were on the margin of the leaf, sometimes on the interior; sometimes on old, yellow leaves, sometimes on vigorous green ones; sometimes in the shade, sometimes in the sun. Always, the under surface (and sometimes, also, the upper surface) was covered with Botrytis. Frequently, at the center of the spot on the upper surface there were to be seen the remains of a Botrytis-infested currant berry which, apparently, had fallen upon the leaf and infected it. Plainly, the brown spots were caused by the Botrytis. The damage done was negligible.

Peck (32, p. 19) has reported the occurrence of *Botrytis plebeja* Fres. on the lower surface of large brown spots on living leaves of currants at Menands, N. Y. Probably, our disease and fungus are the same as his.

During prolonged periods of wet weather the same Botrytis is parasitic, also, upon the green fruit, fruit pedicels and young shoots of red currants.

ANGULAR LEAF SPOT, *Cercospora angulata* Wint. The statement of Hesler and Whetzel (17, p. 219) that this disease is common in New York does not accord with our experience. Specimens of it were received from Highland in 1897 and from Long Island in 1900. Since 1900 we have not seen it. Peck does not mention it in any of his reports.

CRINKLE LEAF. Among red currants there occur, occasionally, plants with crinkled leaves which are more deeply cut and sharply lobed than normal leaves; also, the leaves are somewhat smaller and darker green than normal. Frequently, the epidermis is separated from the parenchyma of the leaf on the under surface. Affected plants, tho not much reduced in size, are markedly less productive than normal plants. Numerous examples of this disease have been observed at Milton, Highland and Fayetteville. At first it was suspected that the affected plants were merely of a different variety from their neighbors; but this idea was given up when it was discovered that different portions of the same bush may show normal and crinkled foliage at the same time. Several cases of this kind have been observed. One-half of a bush at Milton was affected in 1909 and again in 1910; while the other half, springing from the same trunk, bore normal foliage in both seasons. The contrast

in appearance between the normal and affected portions was striking. Affected plants do not die. They may show the disease for several years in succession. Whether affected plants ever recover we do not know. The cause is unknown.

FOMES ROOT ROT, *Fomes ribis* (Schum.) Fr. *Fomes ribis* is a woody, yellowish-brown bracket fungus which grows most often around the crowns of currant and gooseberry plants, but occasionally, also, on certain other shrubs and the trunks of sassafras trees. (36.) In New York, the writer has observed it only on the currant and at the following places: Westbury, Highland (two farms), Fayetteville and Geneva. That the fungus is parasitic on its currant host there can be no doubt; but it is not seriously injurious to it.

The writer once made a thoro study of an affected currant plantation at Highland. The currant plants were of the variety Victoria, a red-fruited variety of *Ribes vulgare*. They had been set eleven years. Many of the plants were affected. The owner stated that he had first observed the fungus the first or second year after the plants were set and every year since. Apparently, it had been introduced on nursery stock. It was confined to a portion of the plantation which had been set with purchased plants. An adjoining portion of the plantation, set at the same time with home-grown plants of the same variety, had not shown a single affected plant. The fungus grew around the crowns of the plants at the surface of the soil and in close contact with the living canes which appeared as if they had grown up thru the mass of fungus. (Plate VII.) Often the mass of fungus was a foot in diameter and clung to the currant canes so tenaciously that it was necessary to use a hammer and chisel to separate them.[10] Yet the plants appeared to be in perfect health. Altho they must have been affected for several years they were quite as large and thrifty as unaffected plants beside them.

Likewise, a currant plant of the variety White Grape, a white-fruited variety of *Ribes vulgare*, growing on the Experiment Station grounds at Geneva, was affected by *F. ribis* during a period of at least four years without apparent injury.

Similar testimony concerning the harmlessness of *Polyporus ribis* (=*Fomes ribis*) has been given by van Hall (43) and McCubbin (24, p. 971).

[10] Host Herbarium Specimen No. 81.

FRUIT DROP. The dropping of currant berries when partly grown is the cause of much loss. This trouble is comparable to the " June drop " in apples. It is widespread and occurs more or less every year. Losses of one-third of the crop are not uncommon. The green berries may drop at any time before they are about three-fourths full grown. In severe attacks the ground underneath the bushes is conspicuously littered with the fallen berries. Invariably the dropping is chiefly from the distal (outward) portion of the cluster. In extreme cases nearly or quite all of the berries of a cluster may fall; and even in mild attacks the ends of the fruit stems are bare.

It is quite generally conceded that weather conditions are, in some way, responsible for fruit drop, but there is much diversity of opinion among fruit growers as to the particular kind of weather which induces it. Probably, anything which suddenly checks the growth of the fruit soon after it is set may cause it to drop; for example, excessive heat, drought, a sudden drop to low temperature, or a long period of cold, rainy weather.

Fruit drop is *not* due to imperfect pollination. Currants are self fertile. Moreover, we have known severe attacks of fruit drop to occur in seasons when the weather conditions at blooming time were ideal for cross pollination. In fact, it appears that an unusually heavy setting of fruit is likely to be followed by an unusually large amount of fruit drop.

The writer has no recommendations to make for the prevention of fruit drop.

HYPHOLOMA ROOT ROT, *Hypholoma perplexum* Pk. While examining a worn-out plantation of red currants at Milton on September 27, 1909, the writer found two bushes each of which had a cluster of mushrooms growing at the base. (Plate VI.) Upon digging up the bushes it was found that the mushrooms were rather firmly attached to the wood of the currant plants just below the surface of the soil. Both plants were living, but each had some dead canes and the wood was dead at the point of attachment of the fungus. The fungus was identified by the late Dr. C. H. Peck as a small form of *Hypholoma perplexum* Pk. While it may have been merely saprophytic on the dead wood the circumstances were such as to warrant the suspicion that it was growing parasitically. In either case it is quite unimportant, having been seen but this once.

POWDERY MILDEW, *Sphaerotheca mors-uvae* (Schw.) B. & C. On red currants, powdery mildew is not uncommon. It occurs chiefly on nursery stock; occasionally, on fruiting plants. Usually, the damage done is negligible; but occasionally slight injury results. It attacks the fruit, leaves and young wood at the tips of the shoots, covering them with a conspicuous growth of mycelium which is white when young and cinnamon brown when mature. The perithecia, which contain but a single ascus each, occur on both surfaces of the leaves and imbedded in the dense, brown mycelium covering the shoots.[11]

Several years ago a currant grower at Milton complained of the condition of some red currant cuttings which he had bought from a neighbor. Specimen cuttings, which he sent for examination in mid-winter, were thickly covered over two or three inches of their tips with the cinnamon brown mycelium of *S. mors-uvae*. The presence of mature perithecia made positive identification of the fungus possible. The tips of the affected cuttings were somewhat shrunken. The sender reported that many of the cuttings were like the samples sent, and he feared that they might not make strong plants. However, his fears appear to have been unwarranted because the growth of the cuttings, when planted, proved to be entirely satisfactory.

On the black currant (*Ribes nigrum*), *S. mors-uvae* occurs only rarely. The writer has found it twice on this host. On October 16, 1912, many yearling plants of black currant in a nursery at Stanley were found to be affected. The mildew was confined to the leaves and wood at the tips of the shoots. The affected leaves were smaller than normal. The mildew on the upper surfaces of the leaves was white; that on the under surfaces, cinnamon brown. On the wood, also, it was cinnamon brown. Perithecia were numerous on both surfaces of the leaves (particularly on the under surfaces) and on the wood.[12]

The following day, in a nursery at Orleans, powdery mildew was found attacking leaves at the tips of shoots of black currant in the same manner as at Stanley. In this case the plants were two years old and of the variety Black Champion.

It is noteworthy that the autumn of 1912 was unusually warm and wet and exceptionally favorable for the late growth of black currants.

[11] Host Herbarium Specimen No. 82.
[12] Host Herbarium Specimen No. 83.

The occurrence of *S. mors-uvae* on black currants in New York has been reported previously by V. B. Stewart (42).

SUNBURN OR SUNSCALD. When a period of very hot weather follows a period of wet weather and rapid growth, the fruit and leaves of red currants are liable to become sunburned. The leaves show large, irregular, dead, brown spots. The ripe berries first become light in color with the appearance of having been scalded. Later, they shrivel.

Sunburn is especially to be feared when the bushes carry a heavy load of fruit. The rapid increase in the size and weight of the berries as they ripen causes the canes to bend outward and downward. Then the tender leaves and berries on the interior of the bush become suddenly exposed to the sun. Should bright, hot weather occur while the bushes are in this condition a large percentage of the fruit may be ruined by sunburn. Such trouble may be avoided, to a large extent, by picking part of the fruit early to relieve the canes of a part of their load.

TIPBURN. Cause undetermined. During August, 1912, the foliage of red currants in the vicinity of Milton was conspicuously affected in a manner not observed before or since. Viewed from a short distance the foliage appeared yellow and brown and the canes partly defoliated. Close inspection revealed anthracnose (*Pseudopeziza ribis*) as the cause of a part of the yellowing of the leaves; but many leaves were of yellowish green color without any anthracnose spots and almost all of the leaves had dead, brown margins exactly like potato leaves affected with tipburn.[13] It was observed that the yellowing and browning of the leaves were most severe on spots of light, dry soil and on low, heavy, poorly-underdrained soil; also, on uncultivated soil as compared with cultivated soil. The weather was dry and the plants were plainly suffering from lack of water. Accordingly, the yellowing and marginal browning of the leaves were suspected of being due to the same cause as potato tipburn, namely, excessive transpiration and lack of water. However, in view of the fact that leaf-hoppers may cause a similar marginal browning of the leaves of potatoes and certain other plants (1), a positive statement concerning the cause of this currant trouble cannot be made. While the writer's notes do not mention leaf-hoppers it is possible that some were present.

[13] Host Herbarium Specimen No. 84.

UNDEVELOPED BUDS. Beach (3, p. 418), writing of the variety Cherry, says: "There is also a noticeable tendency to imperfect buds at or near the ends of the shoots, especially on bearing plants. Sometimes two or three joints near the end of the shoot have no buds." While this condition is, perhaps, more noticeable on Cherry than on other varieties of currants, it is not confined to Cherry. Several other varieties are often affected.

It is best observed at the time the leaves are unfolding in spring. The imperfect buds do not swell like normal buds. Upon dissection they are found to contain green tissue. This shows that they are not winter-injured buds and have not been killed by a parasite. They are merely undeveloped buds; but the cause of their failure to develop is unknown.

WHITE CRYSTALLINE DEPOSIT ON CANES. When currant canes are pruned in the spring and there is a copious flow of sap, a conspicuous white deposit forms on the bark below the wounds made in pruning. Sometimes this deposit forms a smooth, white coating on the bark; at other times it is gathered into little heaps. In either case it has the appearance of a fungus growth for which it is often mistaken. Upon microscopic examination the white deposit is found to consist of long, needle-shaped crystals arranged in hemispherical clusters resembling chestnut burs.[14] As the crystals are soluble in water the white deposit disappears when rain comes. For microscopic examination the crystals should be mounted in glycerin in which medium they are but slowly soluble. Chemical tests made by Mr. A. W. Bosworth, formerly Associate Chemist of the Station, indicate that the crystals are some organic compound of calcium. Certainly, their chemical composition is different from that of the large polyhedral crystals found in the bark of currant canes.

WITCHES' BROOM. Only one witches' broom on currant has been observed. This was a fine specimen found at Fredonia July 28, 1909.[15] It was borne near the tip of a tall cane of red currant which, under the weight of the broom, bent far over toward the ground. The broom consisted of 13 vigorous shoots three to eight inches long and five shorter ones — all of the current season's growth and bearing leaves. It was attached at the base of a living 4-inch

[14] Host Herbarium Specimen No. 85.
[15] Museum Specimen No. 144.

shoot of the previous season's growth. (Plate V.) Altho a careful examination of the broom was made its cause was not discovered.

Lemée (39, p. 433) has figured a witches' broom found on red currants in France. His statement that it was caused by the rust fungus, *Cronartium ribicola,* cannot be accepted without evidence.

YELLOW LEAF. Non-parasitic. It is not uncommon for the leaves of red currants to become pale yellow and fall early in June without showing blemishes of any kind. The affected leaves are chiefly the small, broad-petioled first leaves at the bases of the shoots, altho many full-sized leaves may be mingled with them. This may occur on thrifty bushes as well as unthrifty ones and on bushes of any age. It is similar to yellow leaf of the cherry. Usually, it is unimportant.

Severe drought occurring in midsummer may cause a yellowing of currant foliage which, viewed at a distance, resembles a severe attack of anthracnose. Bushes on thin, dry soil show it most. The absence of brown spots on the leaves distinguishes this trouble from anthracnose. The yellow leaves usually wither before they fall.

ELM.

Ulmus spp.

ANTHRACNOSE, *Mycosphaerella ulmi* Kleb. This disease has been observed several times. It attacks, chiefly, young trees of the English elm (*Ulmus campestris*) in nurseries and Camperdown elms (*U. glabra* var. *camperdownii*) of all ages. In the nursery, it may cause extensive defoliation as was observed by the writer at Brighton, in August, 1900, and at Geneva, in October, 1912. Affected leaves show numerous small, reddish brown spots and irregular, dead, brown areas on their upper surfaces; while beneath, they are thickly dotted with conspicuous heaps of white or cream colored spores. The margins of the leaves turn brown and roll upward.[16]

The parasitic stage of the causal fungus is an imperfect form which is usually given the name *Phleospora ulmi* (Fr.) Wallr.; but Briosi and Cavara (5, No. 98) and Clinton (7, p. 727), who hold that it belongs in the genus Septogloeum, call it *Septogloeum ulmi* (Fr.) Bri. & Cav. However, it having been demonstrated by Klebahn (22) that the fungus possesses, also, an ascigerous, or perfect stage,

[16] Host Herbarium Specimen Nos. 86 and 87.

which he calls *Mycosphaerella ulmi*, the rules of mycological nomenclature require the use of that name for the fungus.

A few observations made by the writer indicate that, in different collections, the spores of the fungus are quite variable in size and shape. As we have found them, they are 3-4-septate, usually quite strongly curved, and measure 34–38 x 5.5–6.5 μ. In No. 157 of Seymour and Earle's Economic Fungi, on *Ulmus fulva*, the spores are 3-septate, straight, and measure 33.5 x 6.3 μ. In No. 648 of Krieger's Fungi Saxonici, on *Ulmus campestris*, they are 3-4-septate, strongly curved, and measure 49.5 x 4.7 μ.

YELLOW LEAF. Non-parasitic. In common with several other kinds of trees, Camperdown elms are liable to become affected with a non-parasitic yellow-leaf disease which causes them to shed a part of their leaves during June. Affected leaves first turn yellow without any spotting, then become brown and shriveled. Usually, they fall before changing color from yellow to brown. Yellow leaf is most severe when a long period of wet weather in May is followed by dry, hot weather in June. Altho the quantity of fallen leaves is often considerable, the health of the trees is not likely to be seriously impaired; but the litter which they make is objectionable.

DYING OF BRANCHES. In 1913, the diseased condition of an eleven-year-old elm tree on the Station grounds attracted the writer's attention. When the tree came into leaf certain branches were dead and bare. Upon inquiry, it was learned that similar dead branches had been pruned out of the top of the tree in previous years. An examination of the dead branches failed to reveal the presence of any insect or fungus parasite which might be held responsible for their death.

Additional observations were made during the succeeding three summers without the cause of the trouble being discovered. Each year the number of dead branches increased until by the fall of 1916 nearly one-half of the tree was dead. (Plate VIII.) Invariably, the branches died during winter — never while the tree was in foliage. The fungi found on the dead branches were *Cephalothecium roseum* Cda., *Nectria* sp., *Sphaeropsis* sp., and an unidentified fungus; but all occurred only in small quantity. Mr. W. O. Gloyer, Associate Botanist, made a fruitless search of the dead tissues for the mycelium of the blister canker fungus, *Nummularia discreta*: and Mr. Parrott,

the Station Entomologist, who carefully examined the dead branches for evidence of insect attack found only a little of the work of the shot-hole borer. Also, it is reasonably certain that there was no defoliation of the branches by insects.

Finally, in September, 1916, the tree was dug out, the roots examined and the trunk and larger branches cut up; but nothing was found which would account satisfactorily for the unhealthy condition of the tree. The roots appeared normal, and the trunk and branches were sound except for incipient heart-rot in the center of the trunk near the base.

Other elm trees of the same age standing on either side of the diseased tree at a distance of thirty feet were thrifty and entirely free from dead branches.

TRUNK INJURY from box "protector." In the summer of 1910 the trunk of an elm tree on the Station grounds was encased in a "protector," consisting of a tight, wooden box twenty inches square by six feet high, to protect it from injury by animals. The tree was a thrifty one with an uninjured trunk ten inches in diameter.

In the spring of 1912 the tree showed pronounced symptoms of ill health. Soon after the leaves started they stopped growing and early in the summer the tree died. Upon removing the box "protector" it was found that the bark was dead over a large portion of the trunk. There were patches and strips of dead bark alternating with areas of live bark. The injury extended from the ground upward to a height of about eight feet. A part of the injury, at least, was a year old, for some of the wounds had healed. The dead bark was permeated by the conspicuous white mycelium of an unidentified fungus. Also, two species of boring insects were working in the bark; but Mr. Parrott, the Station Entomologist, expressed the opinion that they were not responsible for the trouble.

While there is much uncertainty concerning the immediate cause of the injury — whether winter injury, fungus, or borers — the indirect responsibility of the box "protector" seems to be clear. It appears that the close boxing of elm trunks is a dangerous practise. Tree protectors should be so constructed as to admit light and air freely to all sides of the trunk; otherwise they may do more harm than good.

ACKNOWLEDGMENT.

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SPRAYING LAWNS WITH IRON SULFATE TO ERADICATE DANDELIONS.*

M. T. MUNN.

SUMMARY.

Experiments made at the Station during the past eight years demonstrate that dandelions may be eradicated from lawns, at relatively slight expense and without material injury to the grass, by proper spraying with an iron sulfate solution. Ordinarily, four or five applications are required. The first spraying should be made in May just before the first blooming period. One or two others should follow at intervals of three or four weeks; and, finally, one or two more in late summer or fall. During the hot, dry weather of mid-summer spraying should be discontinued because of the danger of injury to the grass. A conspicuous blackening of the lawn which follows each application soon disappears if the grass is growing vigorously. Of the other common lawn weeds, some are killed while others are but slightly injured by the spraying. Unfortunately, white clover, also, is killed. Spraying should be supplemented by the use of fertilizers and the application of grass seed in the spring and fall of each year. With proper management it is necessary to spray only about every third year in order to keep a lawn practically free from dandelions.

The cutting-out method of fighting dandelions is laborious and ineffective unless the greater part of the root is removed. Shallow cutting, unless done frequently, is worse than none at all, because each cut-off root promptly sends up one or more new plants.

Tests of certain after-treatment measures in the form of reseeding, liming of the soil, and fertilization with commercial fertilizers and stable manure, used in conjunction with the spraying operations, gave results which serve highly to recommend their use either singly or in combination on lawns.

A study of seed production in the common dandelion shows it to be parthenogenetic, that is, capable of producing viable seeds without fertilization of the ovules by pollen.

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INTRODUCTION.

From experiments conducted at this Station during the years 1909 and 1910 by G. T. French, then Assistant Botanist, and reported in Bulletin No. 335, the conclusion was drawn that the spraying of lawns with iron sulfate solution to kill dandelions is unlikely to prove successful in New York. The apparent failure to kill large, vigorous plants was explained upon the ground that spraying merely kills the leaves, and therefore has only a starving effect upon the roots in the same manner as frequent cutting. However, a survey of the literature of the subject prior to 1912, the time the experiments discussed in this bulletin were started, revealed the almost unanimous recommendation of the use of iron sulfate solution as an effective means of controlling dandelions in lawns. In 1907, Bolley, in North Dakota, reported the results of experiments conducted between 1896 and 1907. It was stated by this author that a new method of fighting dandelions, namely, spraying them thoroly at stated intervals with a differential spray solution made by dissolving two pounds of iron sulfate in one gallon of water will give success, and that the grass need not be injured. This work was more fully discussed in 1908 and some additional measures of control suggested. Again, in 1909, Bolley reported that on large lawns spraying dandelions with iron sulfate is quite practical and the cost, using field sprayers, is less than for mowing. Note was also made of the fact that the iron sulfate has a beneficial effect upon the grass in the direction of the prevention of certain diseases of blue grass. In Rhode Island, Adams (1909) found that dandelions may be held in check and practically eradicated from lawns by spraying four or five times during the season with a twenty per ct. solution of iron sulfate, but that complete eradication cannot be expected because of the fact that the lawns are reseeded by wind-blown seeds. This investigator reported no injury to the grass when the spraying was done about two days after mowing. Experience demonstrated that the most effective spraying was made in the spring when leaf growth was vigorous and the first buds ready to open. Reseeding of the lawn after spraying was also advised.

According to the report of Pammel and King (1909) two sprayings with a twenty per ct. solution of iron sulfate killed many of the dandelion plants in a plat of lawn at Ames, Iowa. Chickweed, also, was killed.

Moore and Stone (1909) reported satisfactory results in Wisconsin from the use of a twenty per ct. solution of iron sulfate sprinkled on lawns with a sprinkling can. Plants not killed by the solution were sprinkled with dry iron sulfate soon after the spraying. This application killed nearly all the remaining plants and apparently caused no injury to the grass.

In South Dakota, Olive (1909) reported that the results obtained from spraying dandelions with iron sulfate were not as favorable as those reported by other experimenters. However, young plants were killed by one application, while old, large plants required three or more applications. This worker suggested that, possibly, continued use of the spray for two seasons would give better results.

Selby (1910) mentioned the fact that, in Ohio, tests of iron sulfate show that the first spraying should be made before blossoming of the dandelions, and stated that two or three later sprayings would probably be found advisable.

At the time of the preparation of this bulletin for publication a survey of the literature reporting recent work on dandelion spraying shows that nearly all investigators are agreed that iron sulfate has considerable value as a dandelion eradicator. In Canada, Fyles (1913) sprayed dandelions with a solution of iron sulfate, two pounds per gallon of water, and reported that after the third application the weeds were still living altho much of their foliage was destroyed. After the third application the spraying was discontinued since the injury to the surrounding grass was greater than to the weeds. No mention is made of the location of the trials, but it is inferred that they were made on lawns. A little later, however, Howitt (1913) reported that after six sprayings with a twenty per ct. solution (two pounds per gallon) of iron sulfate applied with a knapsack sprayer to plots of lawn containing 168 square feet the dandelions were reduced in number over ninety per ct. Aside from the black discoloration which immediately followed spraying and disappeared in a few days, no permanent injury was caused to the grass. In fact, it was noted the following spring that the grass on the sprayed plats was greener and more luxuriant than on the unsprayed plat. In 1913 a one-eighth-acre plat was sprayed six times with a power sprayer using a solution of similar strength. The results obtained were said to be as satisfactory as those secured with a knapsack sprayer. At least ninety per ct. of the dandelions were destroyed. The method is

recommended for lawns badly infested with dandelions. Reseeding of the lawn is advised as a supplementary measure.

During 1914, Arthur reported that, in Indiana, on one area sprayed with iron sulfate four times in 1913 and three times in 1914, most of the dandelions were killed, but that they again appeared during the next three months and later became quite abundant. On another area, sprayed four times during 1914, approximately one-half of the dandelions were killed. After-treatment measures, in the form of liming the soil and reseeding with blue grass seed, were used in these experiments.

In a recent and very complete bulletin, Longyear (1918) discussed the life history of the dandelion and some methods of eradication used in Colorado. This experimenter concludes that there is no easy, certain method of exterminating the dandelion or of holding it in check for any considerable length of time. However, it may be kept under control by persistently employing one or more of the following methods: (a) careful establishment of the lawn and later heavy reseeding; (b) applying a small amount of gasoline or kerosene to the crown of each individual plant; (c) deep digging of the entire plant; (d) prevention of seed production on the premises; and (e) by spraying infested lawns with a solution of iron sulfate. The last-named method, labor costs considered, proved to be the cheapest and most effective method of eradicating dandelions.

THE DANDELION.

SPECIES.

In New York lawns the common dandelion (*Taraxacum officinale* Weber) with its showy flowers and ragged, mussy foliage is the one generally found. However, the red-seeded dandelion (*T. erythrospermum* Andrz.) occurs occasionally. The red-seeded dandelion is readily distinguished from the common dandelion by its smaller, sulphur-yellow heads, glaucous bracts, more finely cut leaves and bright red or red-brown seeds with very fine grayish-white pappus. The common dandelion has orange-yellow heads, coarsely pinnatifid and bluntly lanceolate leaves, and olive-green or brownish seeds with short hard points. In both species, when the blossom appears, the double row of bracts which encloses the many-flowered head spreads apart and exposes them. The inner involucre closes after

blossoming, and the slender beak of each flower elongates and raises the pappus of capillary bristles while the fruit is forming. The whole involucre, which is a double row of bracts, is then reflexed, exposing the nearly mature naked achenes or fruits with the pappus in an open globular head ready to be widely distributed by the wind.

PROPAGATION.

The dandelion is propagated by seeds. However, when once it has gained possession of the soil it will hold on to it persistently, perpetuating itself by heavy seeding, and also by its large, thick, fleshy, deeply-penetrating roots which resist occasional cutting by sending up new sprouts as discussed later in this bulletin.

PARTHENOGENESIS AND SEED PRODUCTION.

Seed production is an important phase in the life history of the dandelion since it is the one important means of distribution of the plant. At the outset of the work with dandelion eradication a study of the problem of seed production was started. The belief seems to be quite generally prevalent that the transfer of pollen from the stamens to the stigma of the pistil is necessary before seeds can be produced on the dandelion. However, in the common dandelion (*Taraxacum officinale*), at least, pollination is not only unnecessary, but may be, perhaps, altogether unimportant as a factor in seed production. Evidently, parthenogenesis, or apagamous development of the embryo, occurs in this species. As early as 1903, Raunkiaer performed some experiments on several forms of *Taraxacum* by employing a method of castration in which both anthers and stigmas of unopened buds were sliced off with a sharp knife. From the results of his work he announced that dandelions in Denmark are parthenogenetic; that is, they produce fruit freely without fertilization. Juel (1904), and later, Murbeck (1904), followed up the work of Raunkiaer with careful cytological investigations of the tetrad formation in the ovule, and found that parthenogenesis does occur in the genus *Taraxacum*. Murbeck studied *Taraxacum vulgare* which produces abundant, but imperfect pollen, and *T. speciosum* which produces no pollen at all. However, Dahlstedt (1904) stated that, in Belgium, there are two or three species of *Taraxacum* in which pollination seems necessary. Schkorbatow (1910), as a result of his studies of parthenogenesis in the genus *Taraxacum*, stated that

the removal of the anthers of the flowers does not in any way affect the germination of the seeds. According to Ikeno (1910), Handel-Mazzetti, in a monograph of the genus *Taraxacum*, states that hybrids appear among the species of the genus. Therefore, normal fertilization may be expected among certain species of *Taraxacum*. Ikeno (1910) also reported that K. Tanaka, in unpublished results of some work done in Japan during 1908 and 1909 in which Raunkiaer's castration method was used, stated that *T. albidum* formed seeds parthenogenetically while *T. platycarpum* did not. Later investigations by Ikeno confirmed Tanaka's findings in that *T. platycarpum* does not form seeds parthenogenetically, while in other forms of *Taraxacum* both normal fertilization and parthenogenesis occur.

All of the experiments and studies concerning seed production made by the writer and herewith reported were made with *Taraxacum officinale* Weber. During the month of May, in the years 1914 and 1915, a number of dandelion buds were enveloped within parchment paper sacks just before they opened. At the time the heads on the same and adjoining plants had produced seeds the sacks were removed. Seeds apparently normal in size, color, and all other respects were formed on the heads enveloped within the sacks, indicating that parthenogenesis, cross-fertilization between flowers of the same head, or self-fertilization had occurred. Some experiments designed to test the effect of pollen from other dandelion flowers yielded inconclusive results, tho giving some indication that fertilization with pollen may occur.

On May 26, 1919, a considerable number of dandelion plants which still held unopened buds were located. With a sharp razor the stamens and pistils together with the upper portion of the two sets of green bracts were sliced off as close as possible to the ovary, and then the head was enclosed in a parchment paper envelope which was secured upright to a stake driven beside the plant. After a number of plants were treated in this manner a series of buds were castrated in like manner and left uncovered; that is, they were not enclosed in envelopes. On June 5 both the bagged and unbagged heads had produced seeds. The seeds from each head were collected in separate bags and later (on June 11) placed in a germination chamber along with separate lots of seeds from adjoining untreated seedhheads which served as checks. The germination tests of these seeds were made by placing them between sheets of blue blotting paper

in a germination chamber maintained at a temperature of 20° C. for the first 18 hours and 30° C. for the remaining six hours of each day. The results of a single test will be given here. They are as follows:

TABLE 1. RESULTS OF GERMINATION TESTS OF SEEDS FROM CASTRATED AND UNCASTRATED DANDELION FLOWERS.

DATE.	Number of Seeds Germinated.		
	Flowers castrated and bagged.	Flowers castrated but not bagged.	Check.
June 11 Test started			
June 17	5	1	4
June 19	18	1	5
June 23	13	0	1
June 26	6	0	1
June 30	44	14	24
July 5	11	4	11
July 9	5	1	9
July 14	39	19	33
July 19	58	50	55
July 21	10	3	9
Total number germinated	209	93	152
Total number not germinated	761	73	62
Total number tested	970	166	214
Percentage germinated	21	56	71

From the results of these tests it seems quite evident that parthenogenesis does occur in *Taraxacum officinale*. This conclusion is in accord with the evidence produced first by Raunkiaer and later by other investigators. Also, it appears that a certain amount of normal fertilization may occur if one is to base judgment upon the difference in germination percentage between the seeds of check plants and those of unbagged castrated flowers. The lower germination of seeds from bagged flowers as compared with unbagged, castrated flowers may have been due to certain factors or abnormal conditions resulting from bagging.

GERMINATION OF THE SEED.

In germination tests of dandelion seeds collected from lawns it was found that some of the seeds were matured sufficiently to ger-

minate as soon as they begun to leave the plant; and the percentage of germination increased directly in proportion to the degree of maturity. Flower heads cut off with a lawn mower immediately after blossoming failed to produce seeds capable of germination. Therefore it seems safe to conclude that a lawn clipped regularly and at least once a week will not be a source of contamination for clean lawns in the neighborhood. However, some seed heads which lie close to the ground escape the mower blades. These must be removed by hand picking or with a dandelion rake if seed formation is to be entirely prevented.

In the germination tests of the seeds used in the study of parthenogenesis it was found that a quicker response was secured when the range of temperature was greater than that ordinarily used, *i.e.*, when the temperature was permitted to rise to 38° C. for a very short time. Also, it was found advisable to place a small piece of folded blotting paper along with the seeds between the folds of paper to facilitate aeration.

DANDELION ERADICATION EXPERIMENTS.

ERADICATION EXPERIMENTS AT THIS STATION PRIOR TO 1912.

The results of lawn spraying experiments conducted by French (1911), while showing the iron sulfate treatment to be partially successful, did not seem to warrant its recommendation as a practicable method of control. A strip of lawn was sprayed six times during each of two successive seasons, using, the first season, 1.5 pounds, and the second season, 2 pounds of iron sulfate in each gallon of water. While this treatment prevented blooming of the dandelions, and even killed many of the plants, a considerable number remained alive when the experiments were necessarily discontinued at the middle of the second season because of severe injury to the grass. Apparently, the stronger solution used the second season caused considerable injury to the grass as mid-summer approached and the rate of growth of the grass decreased owing to unfavorable weather conditions. In summarizing the results of his experiments with iron sulfate solution, French stated that it was not clear why dandelion spraying should fail in New York when it had been successful elsewhere. He further stated that " the failure of the treatment seemed to be due to the great vitality of the dande-

lion roots." In an attempt to throw some light upon the question of the vitality of the dandelion roots French removed the tops from two plants by cutting just below the crown. This was done successively as soon as new leaves had unfolded for eight times when both plants succumbed to the treatment. French reasoned from these results that if dandelions could withstand six or seven cuttings they could survive as many sprayings, or possibly more, since spraying did not remove the tops as completely as did the cutting.

THE EXPERIMENT IN 1912.

Since the experiments of 1909 and 1910 had not met with the success which seemed to be characteristic of the trials with iron sulfate on dandelions conducted elsewhere, it was deemed advisable to give the method a further test. Work was continued this year by laying off a plat 15 feet wide and 100 feet long in an old lawn which was well sodded over with Kentucky blue grass and red top grass, but badly infested with dandelion plants. This lawn was on a clay loam soil and in all particulars typical of the average lawn in this state.

This strip of lawn was sprayed with iron sulfate seven times during the season and at the rate of approximately 100 gallons per acre, slightly over four gallons of the solution being used on 1500 square feet at each application. The spray solution was applied with a compressed-air sprayer and contained one and one-half pounds of iron sulfate to each gallon of water. The spray was applied at intervals of three weeks for the first three months, or during the vigorous growing season, and approximately once per month during the remainder of the season. The last spraying was made on September 20. The first spraying was made on May 4, at a time when the first blossom heads were opening. After each application of the solution the grass in the sprayed area was badly blackened, and remained unsightly for several days. It was noted, however, that the weather conditions at the time of the application had considerable to do with the extent of blackening and the period of time which elapsed before the normal appearance of the lawn was regained. The blackening of the grass was more marked and was retained longer following the sprayings made in the mid-summer months when periods of dry weather prevailed. It was during these periods when the severest injury to the tips of the grass leaves occurred.

Shortly following the fifth spraying, made late in July, it was noted that the grass in the sprayed area showed a darker green color than did the adjoining unsprayed lawn. This darker green color appeared to be cumulative as the season advanced. At the time of the last spraying, September 20, the sprayed area was very clearly distinguished by the darker green color of the grass (Plate IX, fig. 1), also there were but few living dandelion plants remaining in the plat. The final spraying for the season killed the foliage on all the dandelion plants in the sprayed area to such an extent that less than thirty plants showed any green foliage when the fall season came to a close. As far as the killing of the dandelion plants was concerned, the experiment was a decided success. Experience seemed to indicate that five sprayings or, possibly, fewer would have secured the same results. The sprayings which could well have been omitted were those made during the latter part of July and in August.

THE EXPERIMENTS IN 1913.

PLAT 1.

In the spring of this year, at the active blooming period, the clear sod of the sprayed area of this plat presented a marked contrast to the adjoining untreated area which contained countless numbers of dandelion plants in bloom (Plate IX, fig. 2; also Plate X).

The eradication of the dandelion plants in this plat was so complete that it was unnecessary to spray this season. However, it was evident that the bare spots left by the dead dandelions would soon be reoccupied by other dandelions from seeds unless something were done to prevent it. Clearly, the advantage gained by spraying should be followed up next spring by reseeding with grass seed to cover the bare spots before dandelions could take possession of them. Also, it was evident that the lawn grasses had been slightly injured leaving some of the coarse weed grasses, such as crab grass, more in evidence.

During this season various tests in the form of after-treatment measures were conducted, having in view the hastening of the healing of the scars left by the dead weeds, and the renovation of the turf. These treatments consisted of the use of fertilizers alone, seed alone, and combinations of fertilizer and seed upon sections of lawn

(25x45 ft.) which included both sprayed and unsprayed areas. The following diagram shows the arrangement and treatment of the different sections.

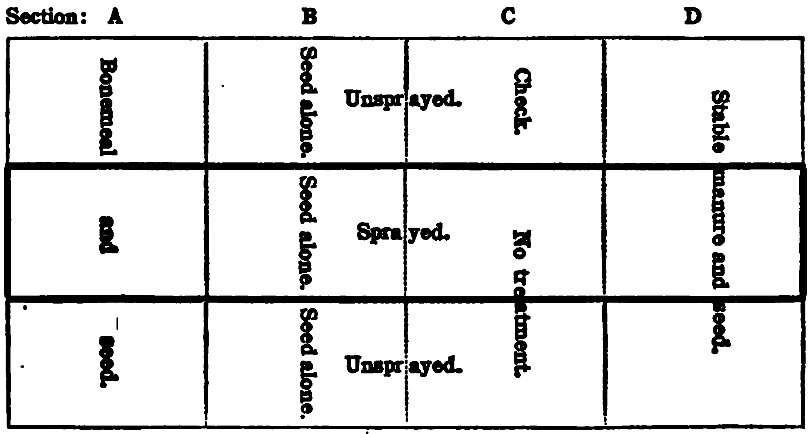

DIAGRAM 1. ARRANGEMENT AND TREATMENT OF SECTIONS IN EXPERIMENT ON AFTER-TREATMENT OF LAWN SPRAYED WITH IRON SULFATE FOR DANDELIONS.

Section A was treated twice during the season (March 29 and October 16) with fine-ground bonemeal at the rate of 1000 pounds per acre.

Section D was given an application of stable manure in December, 1912. This was allowed to remain on the ground over winter and the coarse material raked off late in March, 1913.

On Sections B and C no fertilizer of any kind was used. Sections A, B and D were given a generous reseeding with a seed mixture containing 11.5 pounds of Kentucky blue grass and 8.5 pounds of red top. This amount (20 pounds) allowed 25.8 ounces of seed per square rod of lawn. On April 4, and just before sowing the seed, the four sections were raked over thoroly with an iron rake. After the seed was sown Sections A and B were sprinkled with a light covering of compost soil. The entire area (all four sections) was then rolled with a heavy lawn roller to counteract the "frost lift," and firm the soil about the roots of the grass.

With reference to the results obtained from the after-treatment measures, it was recorded on May 13, 1913, that the application of manure on Section D had retarded, by about two weeks, the

blossoming of the dandelions in the unsprayed area. However, both dandelions and grass made a vigorous growth. Early in July note was made of the fact that, in the sections reseeded, all of the bare spots left by dandelions killed by spraying, were well covered with a growth of young grass plants. The effect of the application of bonemeal on Section A was not evident until early in September when the darker green color of the grass in this entire section became noticeable.

PLAT 2.

Plat 2, on the Station grounds, was staked out early in the spring of 1913. It consisted of an area 15 x 100 feet located north of the Station horse barn, and it crossed, at right angles, the plat sprayed by French in 1909 and 1910. The soil was a clay loam lower in fertility and more responsive to the effects of drought than the soil of plat 1. Like many other soils immediately surrounding dwellings, this soil had been modified by building operations in the past. This lawn was selected because it was believed to be typical in many respects of the average dandelion-infested lawn in this State. The plat selected as well as the adjoining lawn on all sides was badly infested with dandelions. Scarcely a square foot of area could be found which did not contain at least two or three vigorous plants.

This plat was sprayed six times during the season with four gallons of solution made by dissolving one and one-half pounds of the "sugar" form of iron sulfate in each gallon of water. This quantity of solution was found sufficient to cover the area thoroly. After each application the sprayed plat presented the usual black, unsightly appearance which disappeared after a few days.

As midsummer approached it became increasingly evident that this lawn, because of the unthrifty condition of the grass due to low fertility of the soil, would not withstand spraying during the dry midsummer months without some special care in the form of watering and fertilizing. Nitrate of soda has been recommended as a desirable application to induce the growth of grass during the summer months. In order to test the value of this fertilizer in connection with the response desired from spray-injured or blackened lawns, nitrate of soda was applied at the rate of 100 pounds per acre to the west one-half of this plat. For this area (15 x 20 feet) two pounds of the nitrate of soda was dissolved in a large volume of water which was then sprinkled over the lawn with a sprinkling can. Two appli-

cations were made — one on May 24, the other on July 18. The effect of this treatment showed rather promptly in the darker green color of the grass. It prevented, to a certain extent, the brown, parched appearance of the lawn during the months of July and August.

At the time of the last spraying of the season (October 4) there were a considerable number of dandelion plants scattered over the sprayed area. These were nearly all killed by the spraying, tho a few retained some green leaves and gradually recovered, going into the winter with some foliage.

As far as eradication of the dandelions is concerned, the results with this plat were not as marked or as complete as with Plat 1 which was located on a somewhat more fertile soil. The writer believes this to be due to the dry season, the vigorous condition of the dandelion plants (most of them being old and quite large), and the low fertility of the soil which did not give a quick growth to the grass, and cause it to come in where the dandelions were killed out.

THE EXPERIMENTS IN 1914.

PLAT 1.

At the beginning of this season (on May 7, two years after spraying) a count of the dandelion plants showed only twenty-seven in bloom (Plate XI); while the adjoining lawns were golden yellow with blossoms and later white with many fluffy gray seed heads, which served as a great source of infestation for the sprayed area. The result of this infestation was apparent in late summer when it was noticed that a number of young seedling dandelion plants were appearing in the sprayed area. On October 17, at practically the close of the growing season, it was noted that the outlines of the sprayed area were plainly discernible at a considerable distance because of the small number of dandelions present. By the average person this area would be classed as a " clean lawn." Section A, treated with bonemeal during 1913, was still plainly outlined by the slightly greener color of the grass.

PLAT 2.

Plat 2, which was sprayed six times during the previous growing season, showed quite a number of dandelions in bloom (Plate XII).

Fig. 1.

Fig. 2.

PLATE IX.— EFFECT OF IRON SULFATE SPRAY UPON GRASS AND DANDELIONS.

Fig. 1. Showing darker green color of grass on the sprayed area.

Fig. 2. Showing contrast between clear sod of sprayed plat (left) as compared with the unsprayed lawn. Plat 1, 1913.

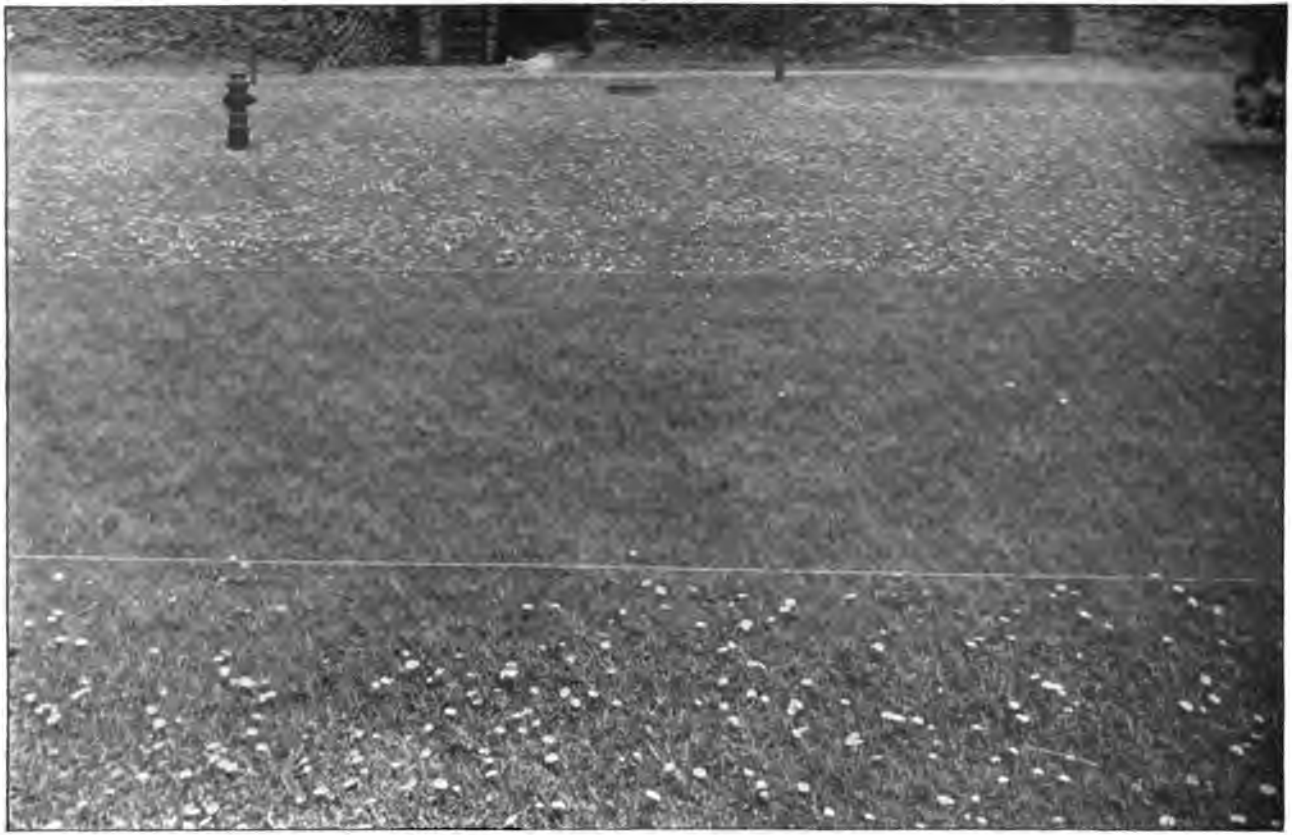

PLATE X.— DANDELION SPRAYING EXPERIMENT.
Appearance of Plat 1 (center) in May, 1913, one year after spraying.

A little later in the spring both of the sprayed plats were plainly distinguishable by the darker green color of the grass, and, in certain areas, by its much larger size. This condition was quote noticeable in the case of Plat 1. The lawns adjoining Plat 2 were badly infested with dandelions. The previous season's experience with this particular plat plainly indicated that on a poor soil with a thin stand of grass and many old, vigorous dandelion plants, attempts to completely eradicate the pest by spraying alone might not be entirely successful. It was apparent that some additional treatment, such as fertilizing and reseeding, should be given the sprayed areas. It was, therefore, deemed advisable to spray this plat for another season and supplement spraying with the above-mentioned after-treatment measures. Accordingly, the dandelions were "sprayed off" at the same time that Plat 3, immediately adjoining, was sprayed. By the term "sprayed off" it is meant that the area was gone over and sprayed with iron sulfate solution only where there were dandelion plants.

PLAT 3.

Plat 3, size 15 x 100 feet, was located so that it immediately adjoined Plat 2 on the north. This plat was badly infested with dandelions, and contained other weeds such as narrow-leaved plantain, mallow, knot grass, and a small patch of wild geranium or cranesbill. By combining Plats 2 and 3, an area 30 x 100 feet was made available for spraying. Beginning May 7, these combined plats were sprayed six times during the season with a solution containing one and one-half pounds of iron sulfate to each gallon. Four gallons of the iron sulfate solution were used at each application. Shortly following the first spraying it was very noticeable that all of the dandelion foliage was killed. However, there were a few plants which were blossoming. The sprayed plats, with these few blossoms, presented a marked contrast to the adjoining unsprayed lawns which were yellow with blossoms (Plate XIII). An observation made during the previous two seasons was again substantiated; namely, that a thoro application of iron sulfate solution early in the spring, when the first central buds are forming and about ready to open, will entirely prevent blossoming and seed formation.

Blackening of the grass followed every application, and the dandelion foliage was killed down. By the end of the season the plats

were nearly free from dandelions. Late in the summer, when rains were more frequent, the sprayed plats took on a darker green color and presented a much healthier and more pleasing appearance than the adjoining unsprayed lawns with the rough, mussy dandelion foliage. Weeds, such as mallow, purslane, dock, and narrow-leaved plantain, which are not as easily killed by the iron sulfate, appeared in the spots where the large dandelions were killed out.

THE EXPERIMENTS IN 1915.

PLAT 1.

On May 7, 1915, three years after this plat was first sprayed, there were only a few (less than 30) dandelion plants in bloom. However, there were quite a number of young seedling plants appearing over the entire plat. At the close of this season, following frequent rains, there were approximately one-fourth as many dandelions in this sprayed area as in the adjoining unsprayed lawn. These plants were mostly young seedlings, and undoubtedly came from wind-blown seeds from plants which seeded heavily in adjoining lawns.

The experiment to date shows quite conclusively that the dandelions can be killed during one season's careful spraying, but that the plants may reappear in the lawn from seeds which are constantly "blowing in" from nearby lawns where the pest is permitted to produce seeds.

PLATS 2 AND 3.

In the spring of 1915 there were comparatively few dandelion plants (less than 100 in the two plats) which blossomed. These presented a marked contrast to the profuse blossoming of innumerable plants in the adjoining unsprayed lawns (Plate XIV). The dandelion plants remaining in the unsprayed plats were single plants, many of them being seedlings. The grass in the sprayed plats showed no injurious effects of the iron sulfate while, on the other hand, it possessed a much greener color than the grass in adjoining areas. In actual practice these sprayed lawn areas would be considered "clean" by the average person, and would receive no special attention.

It was noted at the close of the previous season that certain lawn weeds other than the dandelions were appearing in the bare spots where the latter had been killed out. Some of these bare spots

Plate XI.— Dandelion Spraying Experiment.

Appearance of Plat 1 (left center) in May, 1914, two years after spraying.

Plate XII.— Dandelion Spraying Experiment.
Appearance of Plot 2 (right) in May, 1914, one year after spraying. Compare Plate X.

in the turf were several inches in diameter (Plate XIII). It was because of the presence of these weeds and the few remaining dandelions that these two plats were again combined and " sprayed off " four times during the season with the iron sulfate solution, using one and one-half pounds of sulfate to each gallon of water. No attempt was made to spray the entire area of the two plats, only the dandelion plants and other weeds being sprayed. The usual blackening of the grass followed each spraying, but disappeared after a short time. However, this " spraying off " method has the disadvantage of leaving the lawn streaked or spotted with black for a few days (See Plate XIV). At the time of the third spraying (July 10) it was noted that all of the old dandelions had been killed; but quite a number of young plants were appearing just within the border of the plats. Nearly all of these young plants were within three feet of the border of the sprayed plats, and apparently came from seeds of dandelion plants in the unsprayed lawn adjoining. Following the third spraying, in July, it was observed that because of favorable weather conditions the spray solution was particularly effective in killing the foliage of the weeds. All of the dandelion and narrow-leaved plantain foliage was killed. The foliage of broad-leaved plantain was badly injured. The patch of cranesbill or wild geranium located in Plat 3, also showed serious foliage injury.

During the latter part of the season rains were frequent, causing a vigorous growth of grass, and also forcing the growth of a number of seedling dandelion plants in the sprayed area. The sprayed area, with its darker green grass, presented a much more even and pleasing appearance than did the adjoining unsprayed lawn where the dandelion plants were very thick.

AFTER-TREATMENT OR RENOVATION MEASURES ON PLATS 2 AND 3.

Methods of renovation or after-treatment were started on April 4. Plats 2 and 3 were divided crosswise into four sections or areas each 25 x 30 feet. The following diagram shows the location and treatment accorded each section.

Section A was retained as a check. No treatment.

Section B was treated with 40 pounds of a complete fertilizer having a composition of 3–10–12.

Section C was treated with 50 pounds of ground limestone.

Section D was reseeded with a mixture of equal parts of Kentucky blue grass and red top grass seed which was carefully raked in with a garden rake, especial attention being given to the bare spots in the turf.

Section: A	B	C	D
Check. No treatment.	Complete fertilizer.	Ground limestone.	Heavy reseeding.

DIAGRAM 2. ARRANGEMENT AND TREATMENT OF THE FOUR SECTIONS OF PLATS 2 AND 3 IN EXPERIMENT ON AFTER-TREATMENT OF LAWN SPRAYED WITH IRON SULFATE SOLUTION.

Results of the after-treatment measures were first evident forty days later when the section treated with complete fertilizer was found to be plainly outlined because of the distinctly larger size and darker green color of the grass. The growth of the grass had a tendency to cover up bare spots left by dead weeds. No noticeable results, during this season, came from the use of the ground limestone. In Section D, which was reseeded, there was a satisfactory growth of young grass plants. However, it was about ten weeks before the dead-weed scars in the turf were completely healed over.

THE EXPERIMENTS IN 1916.

In the spring of this year, at the time of the first profuse blooming, the sprayed plats were readily discernible at a distance by the small number of dandelion blossoms as compared with the unsprayed portion of the lawn. The stakes which originally marked the corners of the plats were easily located by laying lines around the border as indicated by the dandelion blossoms. Where the end lines crossed the side lines the corner stakes were found imbedded in the turf.

In order to measure the difference in number of dandelions and express it in numerical terms, a strip ten feet wide was laid off along the entire south boundary of the plats. This area was so located as to include a strip five feet wide by 100 feet long on both the sprayed

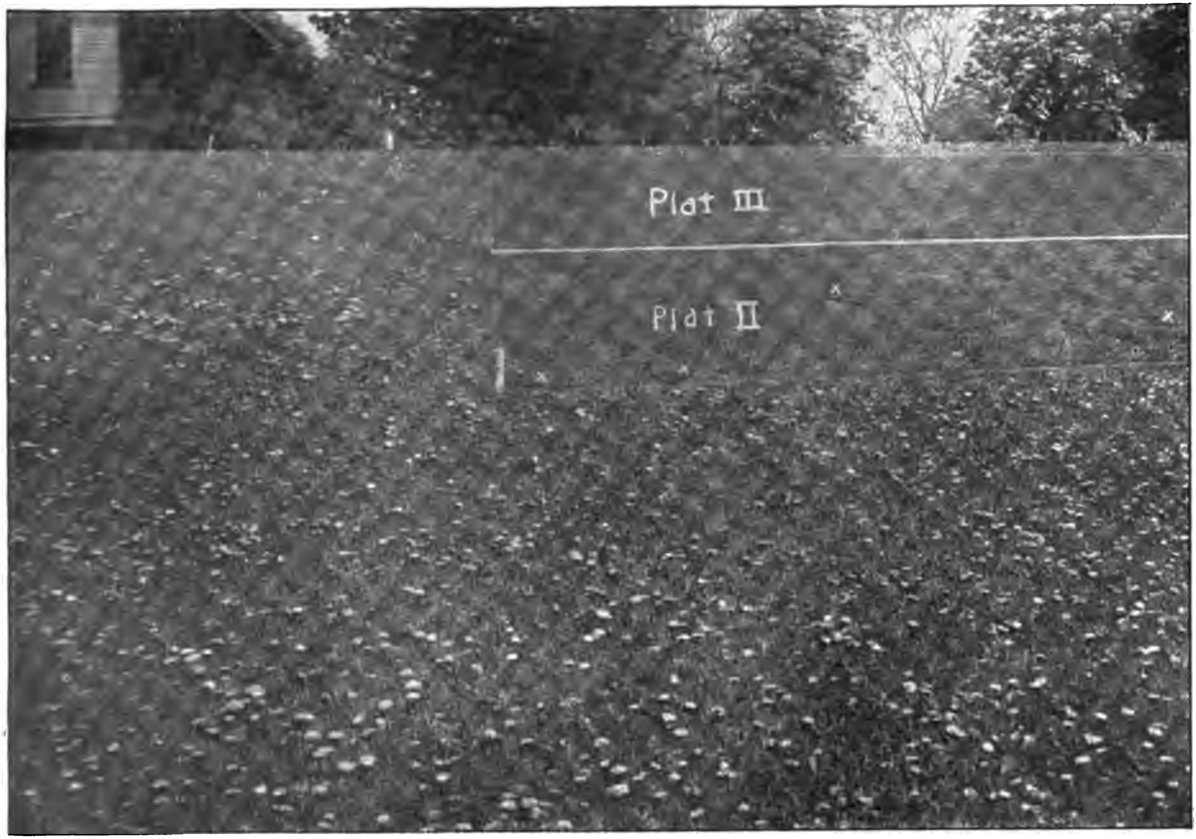

PLATE XIII.— DANDELION SPRAYING EXPERIMENT.

Appearance of Plats 2 and 3 after the first spraying in spring of 1914. The cross marks (×) indicate the location of typical bare spots left by dandelions killed by spraying during 1913.

Plate XIV.— Dandelion Spraying Experiment.

Appearance, in 1915, of Plats 2 (center) and 3 (right) two and one years, respectively, after the first spraying. Note the black streaking resulting from and immediately following " spraying off " dandelions in the spring of 1915.

and the unsprayed lawn. The dandelion blooms were then carefully counted in each strip. The results are as follows:

A strip 5 x 100 feet within the sprayed area contained 140 blooms.

A strip 5 x 100 feet within the unsprayed area contained 1245 blossoms.

In like manner, a strip ten feet wide and thirty feet long was laid off on the east boundary of the two plats, thus including a five-foot strip of unsprayed lawn and an adjoining five-foot strip of sprayed lawn. The counts of dandelion blooms within were as follows:

A strip 5 x 30 feet within the sprayed area contained 72 blooms.

A strip 5 x 30 feet within the unsprayed area contained 628 blooms.

As regards the effectiveness of the spraying, the above figures show that there were approximately 89 per ct. fewer dandelion blooms in the sprayed area. Apparently, the two or three season's spraying on Plats 3 and 2, respectively, had reduced the number of plants 89 per ct. None of the plants remaining on the sprayed area had as yet produced seeds.

THE EXPERIMENTS IN 1917.

CONDITION OF PLAT 1.

At the beginning of this season, five years after spraying, there appeared to be fully as many dandelion plants in the sprayed plat as in the adjoining areas, with the exception of the western section of the plat which was treated with manure in 1913. Here, the effect of the manuring had, apparently, been in the direction of causing a vigorous growth of grass which prevented the reappearance of seedling dandelion plants.

CONDITION OF PLATS 2 AND 3.

On these two plats there were fewer dandelions than on the adjoining unsprayed lawn. However, seedling dandelions were appearing in constantly increasing numbers.

SPRAYING LAWNS WITH A POWER SPRAYER — PLAT 4.

Even tho the work of the previous years with a small outfit on small plats had been comparatively successful, it seemed advisable

to supplement it with a more extensive trial on larger areas with a power sprayer. Accordingly, six areas of the Station lawn located between walks, drives and buildings, and which were badly infested with dandelions were sprayed four times during the season with iron sulfate solution. At each application 300 pounds of the sulfate were used in 200 gallons of water, and applied with a power orchard sprayer equipped with a single lead of hose 80 feet long, a six-foot spray-pole and a nozzle of the Mistry Junior type. With this outfit the pressure was maintained at about 200 pounds, thereby making it possible to apply the solution in the form of a fine, mist-like spray. The long lead of hose and spray pole enabled the operator to reach all parts of the lawn and to do the work thoroly and rapidly.

Four applications of the solution were made, on May 14, June 4, June 28 and July 26. Owing to the fact that the power sprayer was no longer available for this work, the spraying was discontinued in July after the fourth application. Judging from the results obtained in the smaller experiments, later applications would have been desirable and undoubtedly would have increased the effectiveness of the treatment.

The results obtained from the use of the power outfit were similar to those obtained with hand sprayers. The grass was somewhat blackened after each application. The dandelion foliage was quite effectively killed, altho a large number of plants recovered after each application and made a growth of leaves three to four inches long before the next application was made.

Shortly following the first application there was a marked contrast in appearance between the sprayed lawns and some unsprayed lawns nearby. On the unsprayed lawns countless dandelions blossomed and formed seeds which were scattered widely by the wind. The sprayed lawns showed practically no blossoming dandelions and only an occasional large plant still living. Following the fourth spraying, made July 26, and at a time when the lawn soil was comparatively dry, the lawn grasses were badly blackened, and in some places severely injured. This injury to the grass is believed to have been due partly to the dry condition of the soil, and partly to the influence of the light shower which fell during the evening of the day the solution was applied. This experience confirms a previous conclusion, namely, that lawns should not be sprayed with iron sulfate during the hot dry weather of July and August when the lawn soil is dry.

PLAT 5.— THE ROBINSON LAWN.

This test was made on the lawn of Mr. R. H. Robinson in the city of Geneva, which represented well the average lawn surrounding a city residence. The turf, composed of Kentucky blue grass and some red top, was quite badly infested with dandelions. This lawn was sprayed four times during the season of 1915. A barrel sprayer mounted on wheels and equipped with a long lead of hose was used, and the solution was made by dissolving one and one-half pounds of iron sulfate in each gallon of water.

The results of the treatment were very similar to those obtained on the Station grounds. The early blossoming of the dandelions was prevented, and nearly all of the dandelions were killed. The grasses suffered no permanent injury.

One application of the solution was made July 31 when the soil was quite dry. Following this spraying there was quite serious blackening and scorching of the grass in certain spots where the soil was dry and the solution applied in liberal quantities. This experience agrees with a previous one on the Station lawn in which it was clearly demonstrated that lawns should not be sprayed during the dry summer months when both the grass and the soil lacked moisture. Also, some care should be exercised to prevent drenching of the grass since injury to the crowns of the plants may result.

At the end of the season, this lawn showed approximately three per ct. of the original number of dandelion plants, and none had blossomed or formed seeds. This season's test was considered to be successful.

THE EXPERIMENTS IN 1918.

PLAT 1.

This strip of lawn, five years after spraying, had no appearance of any kind which would aid in determining its location. Some white clover and yellow trefoil had reappeared in the plat, and it was well infested with dandelions. There were as many dandelions in this plat as in the adjoining lawns; but it is safe to say that they were younger plants, the leaves being smaller, more nearly erect, and in more compact clusters.

PLATS 2 AND 3.

These plats were in no particular to be distinguished from adjoining lawns. Apparently, they contained as many dandelions. Plants

of narrow-leaved plantain, also some patches of white clover and yellow trefoil were to be found scattered over both plats as well as in the adjoining lawn. With the exception of Section D, which was reseeded with grass seed, the condition of these plats plainly emphasized the great need of after-treatment measures to follow the spraying.

PLAT 4.

The appearance of the lawns included in this plat, one year after the first spraying with the power sprayer, gave unmistakable evidence that the extent of blossoming had been very materially reduced. A large majority of the old plants had been killed. Nevertheless, there were many young plants in certain parts of the sprayed area. In the light of previous experience it was plainly evident that the spraying operations on this plat were discontinued too early in the season to derive the greatest benefit from the treatment. Certainly, the plants should have been forced into the winter without foliage.

PLAT 5. THE ROBINSON LAWN.

In the spring of 1918 a small number of dandelion plants were forming blossom buds, and several had blossomed when the owner made an application of the spray solution. Blooming was thereby prevented and, so far as could be determined, there was no increase in the number of dandelions present in the lawn this season.

THE EXPERIMENTS IN 1919.

PLATS 1, 2 AND 3.

At the beginning of this season, seven, six and five years, respectively, from the time of the first spraying, it was plainly evident that there were as many dandelions in these sprayed plats as in the adjoining unsprayed lawns. It seemed to be plainly demonstrated that, where the sprayed lawns are adjoined by unsprayed lawns infested with dandelions, it will be necessary to resort to spraying about every third season in order to keep dandelions under control.

PLAT 4.

In June of this season, two years after spraying, it was obvious that, in certain restricted areas adjoining buildings or sheltered by shrubbery, the old dandelion plants had been killed out; only an occasional

seedling plant could be found. However, in the greater part of this lawn area, sprayed four times with the power sprayer, there were as many dandelions as in adjoining unsprayed lawns. But it should be stated that the dandelions in the sprayed area were mainly young seedling plants.

PLAT 5. THE ROBINSON LAWN.

The owner of this lawn decided to spray, in 1919, only restricted areas on which the dandelions blossomed. It was unnecessary to spray the entire lawn. This lawn, being in fairly good condition at the outset of the test, responded well to the treatment by filling in the bare places with a growth of grass. It is believed by the writer that heavy reseeding would have been advantageous in this instance, and would have hastened the thickening of the stand of grass. The behavior of this lawn, bordered on both sides by lawns producing dandelion seeds, seemed to demonstrate that, on the average lawn similarly situated, three or four sprayings during the season with iron sulfate applied carefully and at the proper time will practically control the dandelion pest for a period of from two to three years.

OTHER METHODS OF ERADICATION COMPARED WITH SPRAYING.

CUTTING.

Plainly, the problem of eradicating dandelions by cutting them out involves the question of the large amount of reserve food stored up in the root of the plant; also the fact that dandelion roots, when cut, callous over and produce a varying number of adventitious buds upon the upper end portion of the cut crown of the root.

As early as 1873, Caspary, followed by Warming in 1877 and by Wittrock in 1884, demonstrated that the dandelion (*Taraxacum officinale*) and certain other plants have the power to produce sprouts from adventitious buds on their roots when they are cut.

Hitchcock and Clothier (1898) and also Longyear (1918) have fully demonstrated that pieces of dandelion roots even an inch long may produce many new sprouts from adventitious buds on the cut crowns. The former writers found as many as fourteen new sprouts from a dandelion root cut off two inches below the surface of the soil.

Munson (1903) concluded from his study of the behavior of plants of two species of dandelions that, since every top or crown cut off

may send up from one to six new plants, serious injury may occur to the lawn by materially multiplying the number of plants present, and also by frequently disturbing the turf by cutting.

Henderson (1905) also found that cutting off the plants below the ground with a spud only caused them to come up later.

Bolley (1908) stated that cutting tests upon old and young dandelions demonstrated that plants cut three to four inches below the surface readily send up new plants. He objected to the cutting process, also, because of the time and labor required for effective work, and because the turf is too much disturbed when really serious work is done.

At the outset of the work reported in this bulletin the writer had arrived at the conclusion that digging the dandelion from lawns when persistently followed and properly done, is quite effective in destroying large plants. However, it requires much time and patience, and often fails to kill more than a small percentage of the plants. Unless practically all of the root is removed the remaining portion will, in time, send up from one to several sprouts, with the result that the number of dandelions is increased rather than diminished.

In order to obtain more information regarding the behavior of dandelions after being cut, the following experiments were conducted: (1) In the spring of 1912, ten large dandelion plants on a rather unfertile clay soil were carefully marked with stakes. They were uniform in size, and each bore small flower stalks six to eight inches high. On May 18, when some of the flowers were in bloom and others past blooming, the plants were cut off just below the crown. Subsequently, these plants were cut off twice more during the season, the last cutting being made on August 16. None of them were killed. On the other hand, on October 30, at the end of the growing season, there were twenty plants instead of the original ten plants. Only one plant had remained single; all of the others had formed two or three plants from the cut crown. (2) In an attempt to ascertain the effect of deep cutting on dandelions growing under adverse conditions, twenty large plants were selected in a location where the soil was light in texture and quite dry during the greater part of the year. The plants were marked with stakes, and then cut off about three inches below the surface on April 26, 1915. On July 22, following a period of very dry weather which had prevailed since cutting, thirteen

of the cut plants were dead. The other seven had made some growth. By September 1 only four of the twenty plants were alive. Apparently, the plants were killed at the outset by cutting them during dry weather. These results do not agree with those obtained in other tests on more favorable locations, and can be explained only by the fact that the soil upon which the plants grew was exceedingly dry, and the cutting was immediately followed by very dry weather. (3) Cutting plants in midsummer, or later, caused new growth at the expense of reserve food stored in the roots, and gave the best results by either killing the plants or weakening them.

CUTTING VS. SPRAYING DANDELIONS.

At this station, French (1911) concluded from his experiments and observation that spraying did not remove the leaves so completely from treated plants as did cutting, since plants which survived six or more cuttings could withstand as many sprayings and probably more. In order to obtain further data on this point, three experiments were conducted as follows:

Experiment 1. Ten pairs of dandelion plants of practically the same size, and growing within a foot of each other were selected. One plant of each pair was cut off below the crown, while the other plant was " sprayed off " with twenty per ct. iron sulfate solution. These plants were cut off and " sprayed off " on the same date five times during the season, the first treatment being given on May 3. Following each treatment, the " sprayed off " plants were the first to respond by making new growth. These plants usually made a growth of from three to five inches, and had four or five leaves before the cut-off plants were large enough to be again cut off. At the time of the third treatment, only one of the cut-off plants was alive, while four plants of the "sprayed-off" series were living, two of them having made a vigorous growth. At the time of the fourth treatment only two plants in the "sprayed-off" series were living. The foliage of these was again killed down by the sulfate solution. However, on October 3 of that year one of these plants was still living, and made some foliage which was " sprayed off " for the fifth time, after which it did not reappear. This experiment showed that in every case the " sprayed-off " plants were the last to succumb to the treatment, this indicating, in this case at least, that cutting is more effective than spraying.

Experiment 2. In another attempt to compare cutting and spraying, two adjoining plats, each containing 24 square feet, were treated simultaneously by spraying and cutting. On April 27, 187 plants were " sprayed off " in one plat and 100 plants were cut off in another. At the end of the season, October 6, just previous to the fourth treatment, there were 31 plants to be cut off, and 42 plants to be " sprayed off." Even tho the number of living plants in the two plats was about the same at the end of the season, it must be said that the cutting treatment was more satisfactory than spraying, due to the fact that some injury occurred to the grass on the sprayed plat following the spraying in August. In this test the work was necessarily done at a certain stage of the growth of the plants regardless of the weather conditions. Therefore, some injury occurred to the grass in the sprayed plat in August because of dry weather and a dry soil. In practice, such injury may be prevented by withholding spraying in dry weather.

Experiment 3. In another experiment, two adjoining plats, 3 x 6 feet and thickly infested with dandelions, were laid off on the lawn a few feet west of Plat 3 of the larger experiments. One of these plats was sprayed with twenty per ct. iron sulfate solution, and on the same day the dandelions in the other plat were cut off just below the crown. The two plats were treated six times during the season. The first treatment was made on May 6. On May 24, the date of the second treatment, 160 small dandelion plants were counted in the sprayed area, and 45 in the cut-off area. Here, again, following the first spraying, there was a quicker response of the sprayed dandelions than of the plants cut off. The following tabular statement summarizes the results of the various treatments:

	NUMBER OF DANDELIONS.	
	Sprayed Off.	Cut Off.
First spraying May 6	112	108
Second spraying May 24	160	45
Third spraying June 8	107	168
Fourth spraying July 1	96	141
Fifth spraying July 25	39	108
Sixth spraying August 16	25	125
On date of October 3 there remained	3	25

These results do not show a great difference in the rate or completeness of eradication accomplished by the two methods. However, it must be remembered in this connection that the cost of cutting the dandelions per square foot was considerably greater than for spraying them. During this experiment two cases were noted where spraying off had the same effect as cutting off the crown of the plant in that two to four plants started up from a single root where there was only one plant before. This is exceedingly common when plants are cut off or partially dug out.

THE USE OF GASOLINE AND KEROSENE ON DANDELIONS.

Of the various substances recommended as efficient herbicides, gasoline and kerosene are the most common.

Henderson (1905) treated dandelion plants by pouring brine, gasoline, and kerosene upon cut and uncut dandelions, and concluded that of the three substances used gasoline proved most efficient. The other two were not wholly efficient or caused injury to the grass at the point of application.

Olive (1909) treated dandelions by applying sulfuric acid, gasoline, and kerosene to the cut top of the crown. Sulfuric acid of twenty per ct. strength and kerosene were found to be effective. Gasoline, on the other hand, failed to kill the plants completely since the cut roots sprouted in two to three weeks.

Longyear (1918) concluded from his recent work with creosote, gasoline and kerosene that these are all effective herbicides, but that their use involves much time and expense, and some serious injury to the lawn may result where many dandelions are present.

In 1912, a number of large dandelion plants were selected and marked with stakes. By means of an oil-can a small quantity (one-half to one teaspoonful) of gasoline was squirted into the crown of each plant. Seven days later eighty per ct. of the plants were dead. Those which survived the treatment in time regained normal foliage.

In 1914, gasoline and kerosene were similarly applied to marked dandelion plants with the result that a high percentage of the plants were killed with one application. Kerosene proved to be nearly as effective as gasoline. However, the small spot of grass killed around each plant was larger in the case of kerosene than with gasoline, apparently because the kerosene did not evaporate as readily. A patented apparatus called a " dandelion gun " with which the plants

were cut off below the crown and a small quantity of gasoline applied to the cut surface of the root was used with some success. This proved to be slow and expensive work when attempted on anything like a large scale.

The results of the work with these herbicides left but one conclusion, namely, that they are fairly effective, but the cost of application in time and material is relatively great. When a large number of dandelions are present in a lawn, the injury to the turf by the killing of the grass around each plant may be considerable in the aggregate.

OBSERVATIONS BEARING ON THE ACTION OF IRON SULFATE UPON THE LEAVES AND ROOTS OF DANDELION PLANTS.

At the outset, it was recognized that the action of the iron sulfate upon the dandelion foliage is a separate physiological problem requiring considerable study for its solution. This was not attempted in connection with the spraying experiments. However, some observations were made which are herewith presented. Soon after the solution was applied and the foliage began to dry off, the iron sulfate appeared on the plants as a whitish deposit. This was followed by the appearance of small drops of milky juice (latex) on the leaves and flower stalks. Longyear (1918) has recently suggested that this oozing of the latex is due to the absorption of the iron salt by the cells of the leaves and flower stalks, and the production of sufficient internal pressure or turgor to rupture the cell walls and liberate the latex. The writer's views accord with this explanation of the phenomenon. Shortly following the oozing of the latex, the foliage begins to blacken, first at the edges of the leaves and around points of injury. This blackening was invariably hastened by bright sunlight. However, it was noted in two cases that the killing of the plants was hastened by a period of cloudy, showery weather following the application of the sulfate in bright sunlight. Also, by heavy dew or a light shower during the first night after spraying. In addition to the withdrawal of moisture from the dandelion leaves there was plainly a chemical action of the iron sulfate upon the chlorophyll of the leaf whereby it was decomposed into a dark-colored substance scattered thru and within the leaf tissue. This action was observed to be more rapid when the application of the spray was made during

warm weather following a period of rainy weather while the foliage was turgid. The sulfate solution may have a corrosive action upon the cell walls, since its effect was soon evident upon dead or yellowing tissue, injured tissue, and at the mutilated edges of the leaves.

In addition to the action upon the leaf tissue, it was found that, within a certain restricted area on Plat 5 (the Robinson lawn) where the solution at one time was applied too liberally, there was a very noticeable effect upon the crowns of the dandelion plants. Upon digging these plants, they were found to show more or less injury in the form of a decay of the upper portion of the crown. Often the decayed layer was one-half inch thick.

EFFECT OF IRON SULFATE SOLUTION UPON OTHER LAWN WEEDS.

During the progress of the work opportunity was afforded to observe the effect of the solution upon other lawn weeds. In a general way the results can be briefly stated as follows:

Common chickweed (*Stellaria media*) was killed after repeated sprayings.

Purslane (*Portulacca oleracea*) and yellow trefoil (*Medicago lupulina*) were killed.

Wild geranium or cranesbill (*Geranium Robertianum*) and mallow (*Malva rotundifolia*) were badly injured.

Heal all (*Prunella vulgaris*), gill-over-the-ground (*Nepeta hederacea*), broad-leaved plantain (*Plantago major*), curled dock (*Rumex crispus*), and narrow-leaved plantain (*Plantago lanceolata*) were either killed or badly injured.

Knot grass (*Polygonum aviculare*) and crab grass (*Digitaria humifusa*) were either uninjured or were slightly injured.

In the case of some of the weeds listed above, the extent to which they were injured depended upon their age and the thoroness with which they were sprayed. However, it must be remembered that drenching a lawn to kill the weeds may be followed by very severe injury to the grass.

Experience with lawn weeds such as chickweed, crab grass, dock, plantain and wild geranium emphasized the necessity of reseeding the lawn heavily after the last spraying in order to fill up the bare places in the turf where the clumps of dandelions were killed out. Nature appears to abhor bare places, especially in lawns, and often covers them with weeds.

EFFECT OF IRON SULFATE SOLUTION UPON LAWN GRASSES AND CLOVERS.

At the outset it should be stated that the clovers, including yellow trefoil and low hop clover (*Trifolium procumbens*), are killed by spraying with iron sulfate solution. No spraying with iron sulfate on lawns should be attempted unless the owner is willing to sacrifice, temporarily, the white clover (commonly called white dutch clover).

The first effect of the solution upon the lawn grasses is to cause a certain amount of blackening and discoloration immediately following each application. This discoloration lasts for only a few days, after which the grass regains its normal green color. Later, in our experiments, spraying was invariably followed by a more vigorous growth of the grass which was noticeable for at least a part of the first season following spraying, and, in one instance, during the second season. This better growth of the grass was invariably associated with a darker green color which was held during the entire season. The beneficial effect of iron sulfate solution upon grass has been observed and commented upon by other experimenters. Bolley (1909), in reporting it, mentions the fact that in North Dakota the iron sulfate solution had a very beneficial effect in the prevention of certain fungous diseases which attack blue grass.[1]

Aside from the temporary blackening, no serious injury to the grass occured except in one instance, mentioned elsewhere in this bulletin, when the spray solution was applied while the lawn soil was very dry; and in one other case when the solution was applied in too liberal quantity, causing some injury to the grass roots. Fortunately, both of these conditions can be readily avoided by selecting suitable periods for the spraying, and using care in applying the solution. Another form of injury, usually so slight as to be negligible, is that which occurs at the tips of leaf blades recently cut off by the mower or broken by excessive tramping.

TOXIC ACTION OF IRON SULFATE.

As regards the toxic action of iron sulfate upon the roots of dandelion plants in sprayed lawns, the writer will not venture an opinion since the experiments yielded no evidence upon this interesting point.

[1]In this connection it is interesting to note that, in some experiments made by Waite (1910), the addition of iron sulfate to self-boiled lime-sulfur solution sprayed on apple foliage caused the leaves to become darker green and hang on the trees longer.

On Plats 2 and 3, which received excessive quantities of the spray material, no injurious effect of iron sulfate in the soil was detected. In searching for an explanation of this matter, the writer is inclined to accept the view recently advanced by Longyear (1918), namely that iron sulfate is unlikely to produce an injurious effect on the roots of the plants since the chemical oxidizes very readily and, upon reaching the soil, is combined with soil constituents, and becomes insoluble and inert.

In this connection it is interesting to consider the killing of the clover by the sulfate in the light of the work of Ruprecht (1915), who found that sulfate of iron has a very harmful effect upon the roots of clover plants when used in excess of four parts per million in culture solutions.

At the Indiana Experiment Station, Arthur (1914, p. 35) did some work with red clover which led him to the conclusion that, when mixed with the soil, 400 pounds of iron sulfate per acre is the largest quantity which can be used without danger of injury to the clover.

RELATION OF WEATHER CONDITIONS TO EXTENT OF INJURY TO GRASS AND WEEDS.

Most experimenters recommend that the spraying be done on bright, dry days. In general, this appears to be true. However, according to our observation, there are certain exceptions to that rule. Apparently, the weather conditions preceding spraying and the condition of the lawn soil, whether wet or dry, are important factors. During eight years of observation we have found that serious injury to the grass may result if it is sprayed when the soil is dry. In such cases liberal spraying (drenching) materially increases the amount of injury which follows. Heavy dew following spraying was found to facilitate materially the action of the chemical and consequently increase the injury to the dandelions. It was found that, in certain instances, when the lawn was sprayed on a damp, cloudy day following a period of dry weather (Plat 4 sprayed July 28; and Plat 5, the Robinson lawn), the dandelion foliage was quickly and very effectively killed and the grass also displayed an unusual amount of discoloration. It was observed, too, that young seedlings of grass suffered more than older lawns. Bolley (1908, p. 551) has discussed a similar set of conditions encountered in his work in North Dakota on the eradication of weeds in grain fields.

In another instance spraying with the power sprayer was started on what promised to be a clear day. However, just as the job was finished it began to rain, and some rain fell during the evening of that day. The next day was again clear. It was noted that following this spraying the grass was badly blackened, and in some places quite severely injured. The foliage of narrow-leaved plantain and chickweed was almost completely killed by this application.

Our experience, then, teaches that during the greater part of the growing season when dandelions and grass are making a rapid growth, as in spring and fall, it is advisable to select, as a suitable time for spraying, a bright, clear, sunshiny day when there is but little wind and slight probability of rain for several hours. Also, that lawns should not be sprayed during periods of dry weather in mid-summer when the soil is dry and the lawn grasses are inactive. It seems certain that drenching of the turf with the spray solution should not be permitted at any time.

CONCLUSIONS AND RECOMMENDATIONS.

OUTLINE OF LAWN TREATMENT.

Eternal vigilance is the price of a good lawn free from weeds. The proper time to commence the fight against weeds is when the lawn is first made. Care should be taken to secure a thick, thrifty growth of grass at the start. Attempts to establish lawns on poor soil illy-prepared usually fail. After the lawn is established constant care is necessary to maintain it in a thrifty condition which will serve as a protection against weeds.

The measures most frequently used for the eradication of weeds from lawns are: (a) digging them out with a knife or spud; (b) heavy reseeding and fertilization to crowd them out; and (c) the use of chemical sprays to kill the foliage. The last-named method is the cheapest and as effective as any; but complete success requires the use of all three methods and some others.

The dandelion and certain other weeds may be eradicated from lawns, without injury to the grass, by proper spraying with iron sulfate solution. However, the weeds will soon return unless supplementary measures are employed. Unfortunately, there is no escape from the menace of dandelions seeding on adjacent grounds, because one has no control over the premises of his neighbors. Neverthe-

less, the occasional plants which survive spraying should be prevented from seeding by digging them out or by applying gasoline, kerosene, or dry iron sulfate to their crowns. The lawns should be mowed frequently, watered in dry weather, well fertilized, and bare spots reseeded. Whenever the dandelions reappear in considerable numbers it will be necessary to again resort to spraying.

CUTTING DANDELIONS.

Cutting off dandelions below the crown with a knife or spud is not only laborious but ineffective unless practically the entire root is removed or the foliage completely removed several times during the season, so that the plant has no opportunity to store up reserve food in the root. Shallow digging, unless done frequently, is worse than no digging because the root, when cut off, sends up from one to several new plants and the final result is a more profuse growth of dandelions. Deep digging, whereby practically the entire root is removed with a spud, stiff-bladed knife, asparagus knife, chisel, or other special tool, is recommended as a means of removing the few plants which survive spraying.

SPRAYING LAWNS.

Spraying with iron sulfate solution will usually prove effective when carefully, persistently and intelligently done.

NUMBER OF TREATMENTS AND TIME OF MAKING THEM.

Our experiments indicate that at least three (or, usually, five) thoro applications during a season are necessary to eradicate the dandelions from the average lawn in this state. To be the most effective, the spraying should be repeated as soon as the dandelion plant has regained new foliage and just before it is full grown — usually when the leaves are three or four inches long. This forces the plants to use up their reserve food stored in the roots, and eventually starves them. On the Station lawns, which were exposed to dandelion seeds from adjoining untreated lawns, it was found necessary to spray every second or third year, and to supplement the spraying with other control measures.

The time of application appears to be important. In our tests, the best results were secured when the first application was made in early spring after the central blossom buds were formed, but before

blossoming. The first application should be followed by two or three later ones at intervals of three to four weeks during the spring growing season, and one or two others in late summer or fall. The last application should be made late enough in the summer or fall to prevent the plants from recovering before the close of the growing season.

A suitable day for spraying is one on which there is little wind and slight probability of rain for several hours. The sky may be either cloudy or clear. A heavy dew the following night is advantageous. Spraying should be discontinued during periods of drought in midsummer, when the grass is inactive and soil is very dry. Serious injury to the grass may result from spraying at such times. As far as possible, it should be arranged to spray two or three days after mowing and to mow two or three days after spraying.

STRENGTH AND QUANTITY OF SOLUTION, AND MANNER OF APPLICATION.

The spray solution is prepared by dissolving one and one-half or two pounds of iron sulfate (also called copperas and green vitriol) in each gallon of water. The weaker solution appears to be entirely satisfactory, and is probably the one to be preferred. Used at this strength the quantity of iron sulfate required for a single application is approximately 175 pounds per acre or four pounds per thousand square feet of lawn. A gallon of the solution will cover about 375 square feet. Iron sulfate for spraying purposes is usually offered for sale in the granular or "sugar" form, which is readily soluble in water. It is comparatively inexpensive, costing, usually, from one to two dollars per bag of one hundred pounds. Since it corrodes metals, the solution should be prepared in wooden or earthenware vessels.

Experience has demonstrated rather conclusively that the effectiveness of the spray solution upon the dandelions depends, to a considerable extent, upon the manner in which it is applied. The best results are secured when the solution is applied in the form of a fine, mist-like spray well driven down among the foliage. While fairly satisfactory results may be expected when the solution is applied judiciously with a sprinkling can, it is recommended that some form of a spray pump be used. The kind of outfit selected should depend upon the size of the area to be treated. For small lawns a compressed-air sprayer, knapsack sprayer, or good bucket pump

with brass cylinder, and equipped with a fine nozzle will be found satisfactory; while for large lawns a sprayer mounted on wheels is desirable. For very large areas (parks, roadsides, etc.) a power-driven field or orchard sprayer will be found most practical. A lead of hose at least 80 to 100 feet long should be used on the power outfits. In any case, the nozzle should be capable of delivering a fine mist-like spray which will drift evenly over the foliage, and the area should be sprayed evenly, avoiding the drenching of any particular part. One of the new type spray-guns attached to a power sprayer will be found to facilitate the work very materially when it is desired to cover a large area quickly and evenly.

The spray solution should be strained thru a fine strainer or two thicknesses of cheesecloth to remove any particles which would clog the nozzles.

CAUTION.

On stone, cement, metals, and cloth, iron sulfate solution produces a conspicuous yellowish-brown rusty stain which is extremely difficult to remove. Accordingly, care should be taken to avoid getting any of the spray on one's clothing or on sidewalks, building foundations, monuments, curbstones and the like. Even the dragging of the wet hose across stone or cement sidewalks will stain them. In our experiments when working around sidewalks and buildings, we have found it convenient to use a screen made of cloth tacked over a light wooden frame (3 by 6 feet). A helper is required to hold the screen in position and move it from place to place as needed.

After using, the sprayer should be washed out thoroly with clean water to prevent serious rusting. The working parts of the spray pump should be kept well oiled.

AFTER-TREATMENT MEASURES.

Our experiments and experience demonstrate that it is necessary to supplement the spraying operations with at least two after-treatment measures, namely, fertilization and reseeding.

FERTILIZATION.

The fertilization of lawns is essential in order to produce a thrifty growth of grass and dense turf for a protection against the encroachment of weeds. In our experiments, five methods of fertilization,

in the form of surface applications, were tested in conjunction with spraying. Briefly, they are as follows:

(1) Spring and fall applications of bonemeal at the rate of 1000 pounds per acre, the fall application giving the best results.

(2) The application of slaked lime at the rate of 1000 pounds per acre. No noticeable response was secured from this treatment.

(3) The application of nitrate of soda at the rate of 100 pounds per acre in the spring after active growth had begun, and again in summer. This gave good results in the form of increased growth of the grass.

(4) The application of a complete commercial fertilizer in the fall.

(5) The use of well-rotted stable manure applied in the fall, and the coarse material raked off the following spring.

The results seem to indicate rather conclusively that the average lawn will require some form of fertilization to quicken grass growth and heal the turf after the dandelions and other weeds have been killed out.[2] When well-rotted stable manure free from weed seeds cannot be obtained, perhaps the best course to follow would be to use a liberal quantity of complete commercial fertilizer in the fall after spraying and apply ground bone during the following two years.

SEEDING.

The renovation of lawns by heavy reseeding with grass seed or grass seed containing a little white clover, to thicken the turf and crowd out the dandelions, has been reported as having given good success in some cases.

Following the use of the spray solution in our experiments, it was found quite necessary to reseed the scars or bare spots in the turf left by the dead weeds. For this purpose a mixture of equal parts of Kentucky blue grass and red top grass seed was used. This was sown on the sprayed lawn, and well raked into the bare spots, after which a dressing of compost was applied. The success attained by this method seems to warrant the following recommendation: Keep at hand, in a dry place, a supply of grass seed mixture containing equal parts of Kentucky blue grass and redtop grass seed known

[2] The subject of lawn fertilization is fully discussed in U. S. Dept. Agr. Farmers' Bulletin No. 494, which will be sent free from the Department, Washington, D. C.

to be quite free from weed seeds. The two kinds of seed should be purchased separately and mixed at home. The prepared lawn mixtures upon the market are usually of very poor quality. They should never be used unless known by test to be composed of pure fresh seed. The home-mixed seed should be sown on the lawn in the spring (April or May) and again in September following spraying, and well raked into the bare spots left by the weeds. A satisfactory seeding requires five pounds of this mixture for a lot 50 x 100 feet, or one ounce per 100 square feet, at each application. If no spraying is to be done the following season, it is often advisable to add four ounces or more of white clover seed to each five pounds of the mixture. White clover responds quickly and aids in forming a dense growth over bare places where weed seeds may lodge and germinate.

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REPORT

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(See also Report on Inspection Work.)

REPORT OF THE DEPARTMENT OF CHEMISTRY.

CARBONIC ACID AND CARBONATES IN COW'S MILK.*

L. L. VAN SLYKE AND J. C. BAKER.

SUMMARY.

Milk is drawn from the udder into a 100 cc. cylinder so as to fill the cylinder from the bottom upward, thus avoiding mixture with air or loss of CO_2. For the determination of CO_2, 2 cc. of milk is forced from the cylinder into the Van Slyke CO_2 apparatus without loss of CO_2. A 20 per ct. solution of lactic acid is used to free the CO_2 in carbonates.

In the case of 25 samples of milk drawn from separate quarters of the udder, the CO_2 varies from 7 per ct. by volume to 86; the pH value varies (6.50 to 7.16) in a general way with the CO_2 content; with increase of the pH value the general tendency of the CO_2 is to increase while the degree of acidity, as measured by titration decreases. In comparison with other workers, the CO_2 values obtained by us are higher. The CO_2 content of normal milk appears to be about 10 per ct. by volume.

It was found that it is possible to remove CO_2 completely from milk by vacuum exhaustion, provided the milk is spread in a thin layer and agitated.

When milk is pasteurized, the CO_2 content is decreased, but the pH value remains unchanged; but if the CO_2 is completely removed before pasteurization, then after pasteurization the pH value appears to decrease slightly.

CO_2 exists in milk as H_2CO_3 and probably as $NaHCO_3$; the ratio being about one part of H_2CO_3 and two parts of $NaHCO_3$.

The CO_2 tension in milk is calculated to be about equal to 50 to 55 mm. of mercury, in case of a 0.01 N solution, pH 6.6, at 20° C.

INTRODUCTION.

The amount of carbonic acid present in cow's milk has been the subject of several investigations.[1-5] The results reported by these

* Reprint of Technical Bulletin No. 69, June, 1919.

[1] Hoppe, F., *Arch. path. u. anat. Physiol.*, 17:417. 1859.
[2] Setschenow, *Ztschr. ration. Medicin.*, 10:285. 1861.
[3] Pflüger, E., *Arch. ges. Physiol.*, 2:166. 1869.
[4] Thörner, W., *Chem. Ztg.*, 18:1845, 1984.
[5] Marshall, C. E., Sp'l Bul. 16, Mich. Agr. Exp. Sta. 1902.

investigators vary from 1.84 to 7.65 per ct. of CO_2 by volume. It is not necessary to review the methods which have been employed to obtain the CO_2 from milk for measurement farther than to say that they have varied from extraction by means of a vacuum pump to expulsion by heat and have been more or less open to inaccuracy; nor is it important to consider previous methods used in measuring the amount of CO_2.

The results of our work are presented under the following divisions: (I) Determination of CO_2; (II) relation of pasteurized milk to CO_2; (III) the form in which CO_2 exists in milk; (IV) the tension of CO_2 in milk.

I. THE DETERMINATION OF CO_2 IN MILK.

In measuring the amount of CO_2 in milk we have used Van Slyke's method[6] as applied by Van Slyke and Cullen[7] in the determination of CO_2 in blood plasma, with certain modifications required to adapt the method to conditions present in milk.

Our method of procedure will be described under the five heads following: (1) Drawing milk from cow's udder; (2) sampling the milk drawn; (3) determination of CO_2; (4) results obtained with different milks; (5) determination of CO_2 in milk by exhaustion.

1. METHOD OF DRAWING MILK.

In order to prevent loss of CO_2, it is of primary importance to have under complete control the method of drawing from the cow's udder the sample of milk in which CO_2 is to be measured. We have found the following method satisfactory: A silver milking-tube is inserted into the teat and the milk flows readily from the udder through this tube. To prevent the milk coming in contact with the air, it is collected in a 100 cc. cylinder provided with a close-fitting, 2-hole rubber stopper. Thru one hole passes a glass tube extending to the bottom of the cylinder, and this tube is connected at its upper end by rubber tubing with the milking-tube. Passing thru the second hole of the rubber stopper is a short glass tube which serves as an outlet for the air in the cylinder while it is being filled with milk. With this arrangement, the milk flows slowly and quietly into the cylinder without exposure to the air. The cylinder fills from the bottom upward, displacing the air until the milk fills the cylinder completely or even to slight overflowing. If desired, the surface of the milk can be protected from the air by a layer of thin paraffin oil but we have not found this necessary.

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[6] Van Slyke, D. D., *Jour. Biol. Chem.*, 30:347. 1917.

[7] Van Slyke D. D., and Cullen, G. E., *Jour. Biol. Chem.*, 30:291. 1917.

2. TAKING SAMPLE FOR CO_2 DETERMINATION.

It is essential that the samples for the determination of CO_2 be taken from the cylinder as soon as practicable after the milk is drawn from the udder, because, on standing, the rising of the fat globules to the surface changes the composition of the different layers of milk, and also there may be diffusion of the CO_2 from the upper surface if the top of the cylinder is not entirely filled with milk. The best method of taking the sample from the cylinder is the following: One end of a 2 cc. pipette is connected to the tube leading to the bottom of the cylinder. Then one blows into the end of the short tube, forcing the portion of milk last drawn from the udder to flow into the pipette until the milk overflows from the pipette, displacing all the air. The sample thus passes from the lower part of the cylinder into the pipette without exposure to the air, without loss of CO_2, without being subject to appreciable change of pressure and without disturbance of the body of the milk in the cylinder. It has been found that the portion of milk first drawn from the udder and occupying the upper portion of the cylinder does not fairly represent the CO_2 content of the entire milking.

3. DETERMINATION OF CO_2.

The 2 cc. sample of milk is run into the Van Slyke CO_2 apparatus. Only slight modifications are required in the method as described by Van Slyke. We find that 5 per ct. sulphuric acid and other mineral acids coagulate the milk and the coagulum interferes with the operation by clogging the apparatus, making it difficult to remove the CO_2 completely from the mixture. Concentration of such acids greater than 5 per ct. was also found unsatisfactory. As the result of numerous experiments with different acids and concentrations, we find that a 20 per ct. solution of lactic acid obviates all difficulties and meets all requirements, dissolving the casein quickly and completely and giving the same corrections as apply to the use of 5 per ct. sulphuric acid. In all of our work here reported, the amount of CO_2 was determined by absorption with a 5 per ct. solution of NaOH, instead of making a correction for inert gases. The results are the same by either process.

4. RESULTS OBTAINED WITH DIFFERENT MILKS.

In Table I we give the results furnished by the examination of 25 samples of cow's milk, drawn from the individual quarters of the udder. In addition to the amount of CO_2 in the milk, we give in each case the pH value and also the titration value expressed as cc. of alkali required to neutralize 100 cc. of milk to phenolphthalein. The results are arranged in the order of the pH values.

TABLE I.— AMOUNT OF CO_2 IN COW'S MILK.

Per ct. of CO_2 (corrected) by volume	pH value	Cc. of 0.1 N alkali required to neutralise 100 cc. of milk	Per ct. of CO_2 (corrected) by volume	pH value	Cc. of 0.1 N alkali required to neutralise 100 cc. of milk
8	6.50	19.1	9	6.62	17.2
7	6.52	20.0	10	6.63	18.0
8	6.53	18.0	10	6.65	16.6
10	6.54	16.0	12	6.70	15.4
10	6.55	17.2	12	6.80	16.0
8	6.55	18.4	18	6.82	13.0
11	6.57	17.2	14	6.86	14.0
10	6.58	17.8	22	6.90	12.0
10	6.58	16.9	33	6.92	12.0
10	6.58	18.4	24	7.00	10.0
9	6.60	18.2	56	7.05	6.0
10	6.61	16.8	86	7.16	4.0
10	6.62	17.8			

A study of the data embodied in the above table leads to the following statements:

(1) With increase of the value of pH, that is, with decrease of hydrogen ion concentration, there is a general tendency for the CO_2 content of the milks to increase and for the degree of acidity, as measured by titration, to decrease. This is not so marked between the pH values of 6.50 and 6.65 as it is above pH 6.65. Below pH 6.65, the CO_2 content varies between 7 and 10 per ct., while above pH 6.65 it increases somewhat uniformly from 12 at pH 6.70 to 86 at pH 7.16.

(2) The acidity, as measured by titration, varies below p H 6.65 more or less irregularly between 16 cc. and 20 cc. of 0.1 N alkali per 100 cc. of milk, while above pH 6.65, the values decrease quite uniformly from 16 cc. down to 4 cc.

(3) In comparison with the values obtained by other workers, our lowest CO_2 values are about equal to, or higher than, the highest values previously reported. Thus, the highest figure heretofore published is 7.65 per ct. of CO_2 by volume, while most of our values range from 8 up to 56 per ct. in milks which appeared by ordinary inspection to be normal. In the milks examined by us which were known to be normal, the value most frequently found is about 10 per ct. However, our work has not been sufficiently extensive as yet to enable us to indicate positively a general average figure or an average range for normal milk.

5. DETERMINATION OF CO_2 IN MILK BY EXHAUSTION.

It was desirable to ascertain whether it is possible to remove the CO_2 completely from milk for determination simply by vacuum exhaustion. We have found that this can be done by observing certain precautions. The milk must be spread out in a thin layer during the exhaustion. We placed 10 cc. of milk of the usual reaction (pH, 6.5 to 6.65) in a 200 cc. separatory funnel and exhausted this for two minutes, turning the funnel end over end slowly in order to spread the milk in a thin layer over the interior surface of the funnel as completely as possible. Air was then admitted and the exhaustion repeated, after which the determination of CO_2 in the sample was made.[8]

Another portion (100 cc.) of the same milk was then placed in a 200 cc. separatory funnel and inverted. This was exhausted for one hour without any agitation of the milk, after which the amount of CO_2 in the sample was determined. We give below the results of the two experiments.

TABLE II.— RESULTS OF REMOVAL OF CO_2 FROM MILK BY VACUUM EXHAUSTION.

ORIGINAL MILK.		AFTER EXHAUSTION WITHOUT AGITATION.		AFTER EXHAUSTION WITH AGITATION IN A THIN LAYER.	
pH value.	Per ct. of CO_2 by volume.	pH value	Per ct. of CO_2 by volume.	pH value.	Per ct. of CO_2 by volume.
6.54	10	6.57	4	6.66	0
6.86	14	6.92	7	6.98	0
6.92	22	7.00	9	7.06	0

These results make prominent certain points as follows:

(1) The CO_2 of milk can be completely removed by vacuum exhaustion, as shown by the results given in the last column of Table II, provided the milk is agitated and kept in a thin layer, the amount of milk used being small enought to permit control of these conditions. If the amount of milk subjected to exhaustion is not agitated and exposed in a thin layer, the CO_2 is not completely removed, as shown by the results given in the fourth column of Table II. In previous work done by others, in which vacuum exhaustion was relied upon to remove the CO_2 from milk, the removal was incomplete, owing to failure to observe the conditions required for complete exhaustion, as in the case of the results reported by Setschenow[9] and by Marshall.[10]

[8] Cullen, G. E., *Jour. Biol. Chem.*, 30:369. 1917.
[9] Setschenow, *Ztschr. ration. Medicin.*, 10:285. 1861.
[10] Marshall, C. E., Sp'l Bul. 16, Mich. Agr. Exp. Sta. 1902.

(2) The removal of CO_2 from milk results in an increase in the value of pH, that is, a decrease in the hydrogen ion concentration or, stated in another way, the milk becomes less acid, tho to an amount that can not be made appreciable by titration in normal milks. In the first sample in Table II, the original milk, containing 10 per ct., by volume, of CO_2 has a pH value of 6.54, which increases to 6.57 when the CO_2 is reduced to 4 per ct., and which increases farther to pH 6.60 when the CO_2 is completely removed.

II. THE RELATION OF PASTEURIZED MILK TO CO_2.

The observation stated above in the preceding paragraph led us to make a study of some results which we had obtained in another investigation relating to the effect of pasteurization upon the reaction of milk. We had noticed that pasteurization, if properly performed, is without observable effect in changing the hydrogen ion concentration of milk. Our experiment was repeated with the modification that the CO_2 was completely removed from the milk by exhaustion before heating. The two experiments give the following results:

TABLE III.— RESULTS SHOWING EFFECT OF REMOVAL OF CO_2 ON REACTION OF PASTEURIZED MILK.

Before Removal of CO_2.				After Removal of CO_2.			
Before heating.		After heating at 63° C. for 15 minutes.		Before heating.		After heating at 63° C. for 15 minutes.	
pH value.	Per ct. of CO_2 by volume.	pH value.	Per ct. of CO_2 by volume.	pH value.	Per ct. of CO_2 by volume.	pH value.	Per ct. of CO_2 by volume.
6.54	10	6.54	2	6.60	0	6.56	0

In studying the results of these two experiments, we notice:

(1) In the milk in which CO_2 is not removed before heating, the pH value remains the same before and after heating, even tho the percentage of CO_2 is decreased by the heating from 10 to 2 per ct.

(2) In the milk in which CO_2 is completely removed by exhaustion before heating, the pH value decreases from 6.60 to 6.56, that is, the hydrogen ion concentration increases appreciably.

(3) We have not yet carried our work far enough to furnish an explanation of the fact noted, but the inference appears justified

that some chemical change occurs in the milk during pasteurization, which results in an increase of hydrogen ion concentration, when CO_2 is absent, but that in the presence of CO_2 any change in the hydrogen ion concentration is in some way, not yet known, masked or offset by the loss of CO_2 which escapes from the milk during heating.

(4) The decrease of CO_2 in pasteurized milk suggests that the CO_2 content of milk might be made the basis of a method for distinguishing pasteurized from normal milk. We are doing additional work in order to determine the limits of effectiveness of such a method.

III. THE FORM IN WHICH CO_2 EXISTS IN MILK.

It has been generally assumed that CO_2 exists in milk as uncombined carbonic acid. From the fact that the reaction of milk is less acid than that given by a corresponding solution of CO_2 in water, it appears probable that the CO_2 in milk is present in part as carbonic acid and in part as bicarbonate. To determine the proportion of CO_2 existing in milk as carbonic acid and as bicarbonate, two methods are available; viz., (1) by calculation based on the application of the law of mass action, and (2) by direct determination. The results obtained by either of these methods can be regarded as only approximate, owing to the high dilution of CO_2 in milk.

1. CALCULATION BASED ON APPLICATION OF THE LAW OF MASS ACTION.

In a solution containing H_2CO_3 and $RHCO_3$, there exists in accordance with the law of mass action definite quantitative relations between the hydrogen ion concentration of the solution and the relative amounts of H_2CO_3 and $RHCO_3$. These relations are expressed by the following equation:

$C_H = K \frac{H_2CO_3}{\overset{\prime}{C}\overset{\prime}{O}_3} = K \frac{H_2CO_3}{\alpha\ RHC\acute{O}_3}$, in which α is the degree of dissociation of $RHCO_3$ into R^+ and $HC\acute{O}_3$, and K is the ionization constant of H_2CO_3. From the foregoing, we have $\frac{H_2CO_3}{RHCO_3} = \frac{\alpha C_H}{K}$. Therefore, to determine the ratio between bicarbonate and carbonic acid, we need only to know the values of K, C_H and α for $RHCO_3$ (as $NaHCO_3$). According to Michaelis and Rona,[11] K equals 4.4×10^{-7}; the C_H value of average milk is about 0.25×10^{-6}; the value of α is difficult to determine with more than an approximate degree of accuracy under the conditions present in milk, but by a method similar to that of Michaelis and Rona, we obtain a value which

[11] Michaelis, L., and Rona, P., *Biochem. Ztschr.*, 67:182. 1914.

makes the ionization of the bicarbonate in milk about 80 per ct. Applying these values in the equation, $\frac{H_2CO_3}{RHCO_3} = \frac{a\,C_H}{K}$, we have $\frac{0.80 \times 0.25 \times 10^{-6}}{4.4 \times 10^{-7}} = \frac{10}{22}$. This result means that the CO_2 in milk is approximately one part of H_2CO_3 for two parts of bicarbonate, or that one-third of the CO_2 exists as H_2CO_3 and two-thirds as bicarbonate.

2. PROPORTION OF CARBONIC ACID AND BICARBONATE DETERMINED BY EXPERIMENT.

The second method of ascertaining the proportion of CO_2 in milk present as carbonic acid and bicarbonate is based on the isohydric principle. A solution of carbonate containing a molecular concentration equal to that of milk would, if adjusted to the same hydrogen ion concentration, have approximately the same relative proportions of carbonic acid and bicarbonate. Milk is approximately a 0.01 N solution of H_2CO_3. In carrying out the details of our experiment, we dilute 10 cc. of a 0.1 N solution of Na_2CO_3 to 100 cc. with water free from CO_2. Then we add a solution of 0.1 N HCl until the reaction is the same as that commonly found in milk (C_H, 0.25×10^{-6}). This requires 6.6 cc. of the acid. Of the 6.6 cc. of the 0.1 N HCl thus required, 5 cc. is used to change Na_2CO_3 into $NaHCO_3$, leaving 1.6 cc. of 0.1 N HCl to act upon the $NaHCO_3$ and form H_2CO_3. In changing the 100 cc. of 0.01 N Na_2CO_3 solution into $NaHCO_3$, the resulting 100 cc. of 0.01 N $NaHCO_3$ has only one-half the neutralizing power of the Na_2CO_3 solution. Therefore, 1.6 cc. of 0.1 N HCl neutralizes 3.2 cc. of the 100 cc. of 0.01 N $NaHCO_3$ solution, forming 32 cc. of 0.01 N H_2CO_3 and leaving 68 cc. of 0.01 N $NaHCO_3$. These results furnish the ratio, 32 H_2CO_3: 68 $NaHCO_3$, or, approximately, the ratio of 1:2; that is, one-third part of the CO_2 exists in the solution as H_2CO_3 and two-thirds as $NaHCO_3$, a result which is in close agreement with that obtained by the calculation based on the application of the law of mass action.

In this connection it is interesting to note that Marshall[12] states that CO_2 is not completely removed from milk by his method of vacuum exhaustion, owing, as he seems to think, to a slow generation of CO_2 in the milk. This is readily explained by the presence of bicarbonate in milk, which gradually gives up its CO_2 under reduced pressure as a result of the reaction of bicarbonate with some of the salts contained in milk.

[12] Marshall, C. E., Sp'l Bul. 16, Mich. Agr. Exp. Sta. 1902.

IV. THE CO_2 TENSION IN MILK.

The CO_2 tension in milk is about the same as in most fluids of the animal body. Using McClendon's chart[13], we find by extrapolation of values that the CO_2 tension, at 20° C., of a 0.01 N solution, pH 6.6, is approximately equal to 50 to 55 mm. of mercury. It is a matter of interest to notice that the CO_2 tension of blood under the conditions is given by him as 47 mm. Comparing this with the value for milk, one would expect a lower value in blood, because the latter is exposed to air in the lungs and therefore subject to loss of CO_2 by removal.

[13] McClendon, J. F., *Jour. Biol. Chem.*, 30:274. 1917.

CONDITIONS CAUSING VARIATION IN THE REACTION OF FRESHLY-DRAWN MILK.*

L. L. VAN SLYKE AND J. C. BAKER.

SUMMARY.

1. The investigation had for its object the study of the extent and causes of the variation of the hydrogen ion concentration in freshly-drawn cow's milk.

2. In the case of over 300 samples of fresh milk, the pH value was found to vary from 6.50 to 7.20, being under 6.76 in 80 per ct. of the samples.

3. In the case of the milk of 20 cows, it was found that the pH value of the milk from different quarters of the udder varied greatly, but in most cases the variations were not large.

4. The pH value is found to vary with the composition of the milk. In general, with a decrease of acidity, there is a marked tendency toward a decrease in sp. gr., and in percentage of fat, total solids, solids-not-fat, casein and lactose, but an increase in proteins other than casein and in ash and chlorine.

5. These changes in composition are such as would be expected in case blood-serum or lymph were added to normal milk. Abnormal conditions in the udder may cause such addition.

6. Examination of abnormal milks of low acidity, having a pH value above 6.80, indicates that the reaction is accompanied by the presence of large numbers of leucocytes, tho the reaction in such cases may be neutralized by the presence of large numbers of acid-producing streptococci.

7. While the belief in the presence of blood-serum or lymph in such milk is supported by several considerations, a careful test for glucose proved negative.

CONDITIONS CAUSING VARIATION IN THE REACTION OF FRESHLY-DRAWN MILK.

The most sensitive method of measuring the reaction of milk is by measurement of the hydrogen ion concentration. This method has been employed by several investigators[1-7] in connection with the study of milk and it has been utilized in the work here reported.

* Reprint of Technical Bulletin No. 70, June, 1919.

[1] van Dam, W., *Rev. gen. du Lait.* 7:121. 1908.
[2] Allemann, O., *Biochem. Ztschr.*, 65:346. 1912.
[3] Taylor, H. B., *J. Proc. Roy. Soc., N. S. Wales*, 67:II, 174. 1913.
[4] Davidsohn, H., *Ztschr. Kinderheilk*, 9:14. 1913.
[5] Clark, W. M., *Jour. Med. Res.*, 31:431. 1915.
[6] Milroy, T. H., *Pharm. Jour.*, 93:350. 1914.
[7] Foa, C., *Compt. rend. Soc. Biol.*, 59:51. 1905.

The results of previous investigators show cow's milk, when freshly drawn, to have a reaction, expressed in terms of pH values, varying from 6.39 to 6.81, the usual range being between 6.50 and 6.65.

It appeared desirable to us to ascertain the extent of variation of reaction in large numbers of milks obtained directly from cows and to learn further, if possible, the causes of such differences. The results of our work will be discussed under the following points: (1) extent of variation of reaction in cow's milk, (2) variation in different quarters of the udder, (3) relation of reaction of milk to composition, and (4) effect of abnormal conditions in the udder.

EXTENT OF VARIATION OF REACTION IN COW'S MILK.

In undertaking to establish in our own experience the extent to which the reaction of fresh milk varies, we measured the hydrogen ion concentration in over 300 samples obtained from two herds of cows; one was a herd of Jerseys and the other of Holstein-Friesians. The samples used in our work were drawn separately from the individual quarters of the udder and the reaction was determined in each. In obtaining the samples, the first few streams of milk were thrown away and then enough more was drawn for use in our experiments. The results do not, therefore, represent the complete milkings of the entire udder but only the foremilk of each quarter of the udder. The use of milk from individual quarters of the udder gives a greater variation in results than would be found if we used only samples representing the mixed milk of a complete milking of the entire udder. The use of the foremilk is very convenient and is justified, since in our experience the reaction of milk rarely changes appreciably in case of normal milks in portions successively drawn during milking provided there is no excessive disturbance of the udder.

In Table I we give summarized results of our examination of over 300 samples of milk, the reaction being stated in terms of pH values.

TABLE I.— RESULTS GIVING VARIATION OF REACTION IN FRESH MILK.

		pH 6.50 to 6.60	pH 6.60 to 6.68	pH 6.68 to 6.76	pH 6.76 to 6.84	pH 6.84 to 6.92	pH 6.92 to 7.00	pH 7.00 to 7.20	Total No. of samples.
Herd I	No. of samples	78	35	23	7	6	2	2	153
	Percentage of total	51	22.9	15	4.6	3.9	1.3	1.3	...
Herd II	No. of samples	61	32	27	15	9	6	5	155
	Percentage of total	39.4	20.6	17.4	9.7	5.8	3.9	3.2	...

The samples of milk from the two herds of cows show quite as wide a range of hydrogen ion concentration as we have found in our entire experience up to the present time, working with a great variety of milks, tho it is probable that somewhat wider variations may occur. Our results indicate that the reaction of fresh normal milk, expressed in terms of pH values, lies between 6.50 and 6.75 or 6.80. In herd I, 136 samples or nearly 90 per ct. of all the samples, and in herd II 120 samples or over 77 per ct., are below pH 6.76, or an average of 83 per ct. of all samples examined. These figures are in agreement with results previously published, except that we find a small proportion of milks which are less acid, the pH values reaching as high as 7.2. We usually find that milks of such abnormally low acidity are sufficiently normal in appearance to pass ordinary market inspection when mixed in the commercial supply with other normal milks. The wide range of values obtained by us is doubtless due to the fact that the samples of milk used in our work represent individual quarters of the udder and not the complete mixed milk drawn from the entire udder at one milking. The mixed milk of a herd shows still smaller variation than that from single cows.

VARIATION IN REACTION OF MILK IN DIFFERENT QUARTERS OF THE UDDER.

It is a matter of interest to show at this point to what extent milk drawn from different quarters of a cow's udder may vary in reaction. In Table II we give results obtained with 20 cows. The hydrogen ion concentration in these samples was determined approximately by means of an indicator (brom-cresol purple) as described in Technical Bulletin No. 71 of this Station. The results are given in two forms. The range of pH values from 6.50 to values above 7 is divided into seven groups, indicated by number, and after each such group number there is given in parenthesis the corresponding range of specific pH values.

A study of the tabulated data suggests the following points of interest:

1. The reaction found to be the more common is that showing the more acid. Out of the 80 samples of milk drawn from the quarters of the udders of the 20 cows used in the work, 39, or nearly 50 per ct., are in group 1, showing the highest acid reaction (pH, 6.50–6.60); 21, or over 26 per ct., are in group 2 (pH, 6.60–6.68); 14, or over 17 per ct., are in group 3 (pH, 6.68–6.76). These three groups contain 92.5 per ct. of the total. Group 4 (pH, 6.76–6.84) contains 3 samples, while the least acid groups, 5, 6 and 7 contain only one sample each.

2. These results indicate that the hydrogen ion concentration of normal mixed milk, when fresh, is that representing the most acid

reaction found by us (pH, 6.50–6.60), the variations all being in the direction of decreased acidity.

TABLE II.— RESULTS SHOWING VARIATION IN REACTION OF MILK DRAWN FROM DIFFERENT QUARTERS OF UDDER.

No. of cow.	Quarter of udder.							
	Right front.		Left front.		Right hind.		Left hind.	
	Group	pH	Group	pH	Group	pH	Group	pH
16	1	(6.50–6.60)	1	(6.50–6.60)	1	(6.50–6.60)	1	(6.50–6.60)
19	1	(6.50–6.60)	1	(6.50–6.60)	1	(6.50–6.60)	1	(6.50–6.60)
20	1	(6.50–6.60)	1	(6.50–6.60)	1	(6.50–6.60)	1	(6.50–6.60)
14	2	(6.60–6.68)	2	(6.60–6.68)	2	(6.60–6.68)	2	(6.60–6.68)
2	2	(6.60–6.68)	1	(6.50–6.60)	1	(6.50–6.60)	1	(6.50–6.60)
13	1	(6.50–6.60)	2	(6.60–6.68)	1	(6.50–6.60)	1	(6.50–6.60)
15	1	(6.50–6.60)	3	(6.68–6.76)	1	(6.50–6.60)	1	(6.50–6.60)
10	3	(6.68–6.76)	2	(6.60–6.68)	2	(6.60–6.68)	2	(6.60–6.68)
12	5	(6.84–6.92)	3	(6.68–6.76)	3	(6.68–6.76)	3	(6.68–6.76)
1	2	(6.60–6.68)	2	(6.60–6.68)	1	(6.50–6.60)	1	(6.50–6.60)
5	2	(6.60–6.68)	1	(6.50–6.60)	1	(6.50–6.60)	2	(6.60–6.68)
9	3	(6.68–6.76)	3	(6.68–6.76)	1	(6.50–6.60)	1	(6.50–6.60)
4	1	(6.50–6.60)	1	(6.50–6.60)	2	(6.60–6.68)	3	(6.68–6.76)
8	2	(6.60–6.68)	2	(6.60–6.68)	1	(6.50–6.60)	3	(6.68–6.76)
6	2	(6.60–6.68)	3	(6.68–6.76)	1	(6.50–6.60)	2	(6.60–6.68)
17	3	(6.68–6.76)	2	(6.60–6.68)	3	(6.68–6.76)	1	(6.50–6.60)
11	4	(6.76–6.84)	1	(6.50–6.60)	3	(6.68–6.76)	1	(6.50–6.60)
18	1	(6.50–6.60)	1	(6.50–6.60)	4	(6.76–6.84)	3	(6.68–6.76)
7	2	(6.60–6.68)	1	(6.50–6.60)	1	(6.50–6.60)	6	(6.92–7.00)
3	1	(6.50–6.60)	2	(6.60–6.68)	7	(7.00–7.20)	4	(6.76–6.84)

3. Comparing the different quarters of the udder in individual cows, we find that there are only four (Nos. 16, 19, 20, 14) in which the reaction is the same in all quarters; and in these cases the reaction is that of the most acid groups, 1 and 2. In another examination of the same 20 cows, 10 individuals were found to give a uniform reaction in the milk from all quarters, eight showing pH 6.50–6.60, and two, pH 6.60–6.68. In Table II the milk of only five animals (Nos. 12, 11, 18, 7, 13) departs markedly from the normal, and in these cases the abnormal condition appears in only six of the 20 individual quarters.

4. While it is not the purpose of this article to discuss in detail the causes of these observed variations of reaction in the milk from the different quarters of the udder, we may state in passing that such variations must be due to some physiological condition of the animal, either a specific bacterial infection of the udder or a more general constitutional condition, such as variation of base and acid relations in the blood stream.

RELATION OF THE REACTION OF MILK TO THE COMPOSITION.

Attention has been called to the fact that the reaction most commonly prevalent in freshly-drawn milk is the one that is most acid, and that the variation is all in the direction of decreased acidity; and, further, that the number of samples in which the acidity decreases is found to fall off rapidly with the greater decrease of acidity.

It was desired to ascertain, if possible, some of the conditions under which decrease of acidity occurs. Attention was turned first to a study of possible relations that might exist between changes in reaction and changes in composition of milk. We made analyses of several samples of freshly-drawn milk which varied noticeably in reaction. Each sample of milk was drawn from one quarter of the udder, the entire contents of the quarter being drawn, except in certain cases as noted. The samples were all from cows whose milk was going into the local market supply. There was nothing abnormal that was observable about the appearance of the milk or the cows, except that the milks with the least acid reaction had the characteristic bluish appearance of what we commonly call "thin" or "poor" milk.

The results are given in Table III, the analyses being arranged in the order of pH values, beginning with the most acid.

TABLE III.— RESULTS SHOWING COMPOSITION OF MILK IN RELATION TO REACTION.

No. of cow.	pH values.	Total solids.	Fat	Total proteins.	Casein.	Proteins other than casein.	Sugar.	Ash.	Chlorine.	Specific gravity.
		Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	
1	6.53	12.81	4.50	3.21	2.55	0.66	4.60	0.70	0.09	1.029
2	6.56	12.07	3.60	3.32	2.56	0.76	4.60	0.65	0.12	1.031
3	6.58	14.57	5.40	3.60	2.75	0.85	5.10	0.72	0.10	1.032
4*	6.60	13.30	3.80	3.31	2.62	0.69	5.20	0.69	0.11	1.030
5	6.70	12.10	3.60	3.21	2.50	0.71	4.40	0.86	0.13	1.030
6†	6.80	13.29	4.30	4.49	2.84	1.65	3.70	0.71	0.14	1.028
7	6.85	13.74	5.40	3.00	2.20	0.80	4.50	0.81	0.14	1.030
8	6.91	9.41	1.20	3.02	2.05	0.97	4.16	0.79	0.14	1.030
9	6.95	10.58	2.40	3.14	2.05	1.09	3.80	0.83	0.16	1.030
10	6.96	10.48	3.40	2.45	1.69	0.76	3.80	0.83	0.14	1.026
11	6.98	10.41	2.80	2.80	1.79	1.01	4.00	0.81	0.15	1.027
12‡	7.00	12.42	5.65	2.73	1.63	1.10	3.20	0.84	0.18	1.022
13	7.04	10.10	2.20	3.23	2.26	0.97	3.70	0.84	0.16	1.030
14[1]	7.06	8.85	2.80	2.64	1.50	1.14	2.60	0.91	0.22	1.021
15	7.15	9.13	2.10	3.14	1.71	1.43	3.00	0.89	0.21	1.026

* Sample was last portion of milk drawn from the udder (strippings).
† Sample was from cow in the last stage of lactation, being nearly "dry."
‡ Sample was from cow just beginning period of lactation, or "fresh" in milk.

An examination of the data in Table III suggests some points of correspondence between the reaction of milk and the composition.

1. We observe that with an increase of pH values in freshly-drawn milk, that is, with a decrease of acidity, there is a marked tendency toward a decrease in the specific gravity and in the percentage of the total solids, fat, solids-not-fat, casein and sugar; but, on the other hand, an increase in albumen and proteins other than casein, and in the ash and also in the chlorine. There is, further, as we have already pointed out in Tech. Bul. No. 69 of this Station, an increase in CO_2 content with decrease of acidity.

2. These findings raise the question as to whether there is any reason for the correspondence existing between the observed changes in reaction and composition. These changes are such as would be expected, if we were to add blood-serum or lymph to normal milk and they are also in agreement with the results reported by others[8-16] who have worked with milk from diseased udders, tho our samples were from udders that were apparently in normal condition. This phase of the question brings us to a consideration of abnormal conditions of the udder in relation to the reaction of milk.

THE REACTION OF MILK IN RELATION TO THE PRESENCE OF LEUCOCYTES AND BACTERIA.

The samples of milks used by us were all from udders which apparently were in a condition of normal health, under casual observation; but a special examination of those samples showing pH values above 6.70 was made for leucocytes and streptococci. For the work done in making these examinations, we are under obligation to Miss Mildred A. Davis, City Bacteriologist of Geneva. Use was made of Breed's method of direct counting in the milk.[17] The results of the work are given in Table IV.

The results in Table IV indicate in a general way that decreased acidity in fresh milk is related to infection of the udder. Decrease of acidity is shown to be associated in a general way with increase of leucocytes, provided acid-producing streptococci are not present in sufficient numbers to neutralize such effect. Thus in sample No. 7, we have a milk not far from normal in reaction, even tho it contains a large number of leucocytes (20 million per c. c.), a number which in samples 14 and 15 gives a marked relative decrease in acid reaction;

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[8] Storch, N., *Jahresb. Thierch.*, 14: 170, 1884, and 19: 157. 1889.
[9] Hoyberg, *Ztschr. Fleisch. u. Milchhyg.*, XXI: 133. 1911.
[10] Fetzer, L. W., *Eighth Internat. Cong. App'd Chem.*, 19: 111. 1912.
[11] Chretien, M., *Hyg. viande et lait.*, 6: 382. 1912.
[12] Allemann, O., *Milkwirtsch. Zentr.*, 44: 122. 1915.
[13] Zaribnicky, F., *Arch. Wiss. u. Prakt. Thierheilk.*, 40: 355. 1914.
[14] Henderson, J. B., and Meston, L. A., *Chem. News*, 110: 275, 283, 1914, and 111: 51. 1915.
[15] Bahr, L., *Ztschr. Fleisch. u. Milchhyg.*, 24: 251, 288, 370, 398, 472. 1914.
[16] Foa, C., *Compt. rend.*, 59: 57, 1905.
[17] Breed, R. S., N. Y. Agr. Exp. Sta. Tech. Bul. No. 49. 1916.

but this condition in sample 7 appears to be accounted for by the relatively large number of acid-producing streptococci (1 million per c. c.).

TABLE IV.— REACTION OF MILK IN RELATION TO LEUCOCYTES AND STREPTOCOCCI.

No. of cow.	pH value.	No. of leucocytes per cc.	No. of streptococci per cc.
		Million	*Million*
6	6.80	1	0.1
7	6.85	20	1.0
8	6.91	3	few
9	6.95	5	few
10	6.96	10	few
11	6.98	8.4	few
12	7.00	4	0.2
13	7.04	3	few
14	7.06	20	0.5
15	7.15	21	0.3

A further study was made of five samples of milk, in which the approximate pH value was determined by means of brom-cresol purple in which Miss Davis made a microscopic examination for leucocytes and streptococci. The results are given in Table V.

TABLE V.— RESULTS OF MICROSCOPIC EXAMINATION OF MILK.

Sample No.	Approximate pH value.	Results of microscopic examination by Breed's method.	
1	6.68–6.76	Many leucocytes. Streptococci innumerable, separate and in clumps at bottom of tube.	Marked acid production by clumps at bottom of tube within 1 hour after milking.
2	6.72–6.80	5 million leucocytes per cc. Streptococci innumerable, separate and in clumps.	Marked acid production by clumps at bottom of tube within 30 minutes after milking.
3	6.76–6.84	Many leucocytes. Streptococci innumerable, separate and in clumps at bottom of tube.	Marked acid production by clumps at bottom of tube within 30 minutes after milking.
4	7.00+	Leucocytes innumerable; few streptococci.	No acid production apparent.
5	7.00+	Leucocytes innumerable; few streptococci.	No acid production apparent.

Summarizing the results embodied in Table V, we notice:

1. In the samples of milk (4 and 5) containing garget, the acidity was least, and these samples contain innumerable leucocytes but few

streptococci. These samples furnished no evidence of increase of acidity on standing.

2. In the samples (1, 2 and 3), which were the more acid ones, there were immense numbers of streptococci, especially in clumps; and while there were also many leucocytes, there was an increase of acidity within a short time after the milk was drawn from the udder.

3. It appears highly probable that the greater acidity in the milks containing enormous numbers of streptococci is due to the formation of acid by these organisms, especially in view of the fact that milk from diseased udders, containing large numbers of leucocytes and few streptococci, shows the lowest degree of acidity found in milks. In other words, we find milks that contain large numbers of leucocytes and are abnormally low in acidity when streptococci are present in only small numbers or entirely absent, usually show appreciably higher acidity when streptococci are present in large numbers.

A SUGGESTED EXPLANATION OF THE DECREASED ACIDITY IN CERTAIN NORMAL MILKS.

We have already stated that the correspondence existing between the observed changes in reaction and composition of milk may be accounted for on the supposition that the change in the direction of decreased acidity is due to the presence of blood-serum or lymph. Decrease of acidity in fresh milk is observed in case of diseased udders and may, therefore, be due to the direct filtration of blood-serum or lymph into the lumen of the alveoli without transformation by the gland cells, or thru lesions caused by bacterial action. This view harmonizes with several facts: (1) It is in harmony with the changes in composition of the milk; (2) it is in agreement with the hydrogen ion concentration shown by normal milk (pH 6.50–6.60) and that shown by blood-serum (about pH, 7.6); (3) it harmonizes with the variation found by us in the CO_2 content of milk, normal milk containing about 10 per ct. by volume, and serum, 65 per ct.; and (4) it is in agreement with the increasing number of leucocytes found in the less acid milks.

Further proof of the presence of blood-serum or lymph in most of the abnormal milks (pH, 6.90–7.20) examined by us is the existence of fibrin in such samples, as shown by Doane's method.[18]

Another method of proof was undertaken, which was to ascertain if glucose is present in the abnormal milks under discussion. If serum passes unchanged into milk, glucose should be present in such abnormal samples in appreciable amount. We were, however, unable in any case to find the slightest trace of glucose in these abnormal milks. If glucose is absent from the serum present in

[18] Doane, C. F., Maryland Agr. Exp. Sta. Bul. 102. 1905.

these milks, the glucose in the blood-serum must be changed into another compound by some agent present in the milk in the udder, which might be udder cells or some enzym. The point calls for further investigation.

The method used by us for the detection of glucose is the following: Proteins are precipitated by 70 per ct. alcohol and the filtrate evaporated to dryness. This residue is extracted first with ether and then with hot 95 per cent. alcohol. The alcoholic extract is evaporated to dryness and the residue again extracted and the extract evaporated to dryness and the residue extracted with a small amount of ether. This residue is used for an osazone test. Glucose added to milk can be easily recovered by this method.

A METHOD FOR THE PRELIMINARY DETECTION OF ABNORMAL MILKS.*

J. C. BAKER AND L. L. VAN SLYKE.

SUMMARY.

The method is based upon the use of a dye called brom-cresol purple as an indicator. One drop of a saturated water solution of this dye is mixed with 3 cc. of milk and the color is observed. Normal fresh milk produces a grayish-blue color. The production of a darker or lighter color awakens suspicion in regard to the normal character of the milk. The color is made lighter by acids, acid salts, formaldehyde, and also by heating above the usual point of pasteurization. The color becomes deeper blue in the case of milk from diseased udders, watered milk, skimmed milk and milk containing alkali or alkaline salts. In the inspection of milk, a sample is taken for further detailed examination in the laboratory if the color is sufficiently lighter or darker than normal to indicate the probability of some abnormal condition.

The method has been applied and results are reported in case of the examination of 570 samples of market milk. Watered milk was detected and also milk containing excessive numbers of leucocytes. A standard of colors can be prepared by which comparison can be made and conclusions more easily reached as to the normality or abnormality of samples examined.

A METHOD FOR THE PRELIMINARY DETECTION OF ABNORMAL MILKS.

In the official inspection of market milk, the primary object is to detect samples that are abnormal in composition as well as in sanitary character. The full examination of a large number of samples for the purpose of detecting an occasional abnormal one involves a relatively large amount of inefficiency in attaining the object. Attempts have been made to minimize the labor of inspection by using some quick and simple method which would serve the purpose of enabling one to detect suspicious samples; and only those samples which show some evidence of abnormality by such preliminary test are selected for further detailed examination in the laboratory in order to confirm or disprove the suspicion. In

* Reprint of Technical Bulletin No. 71, June, 1919.

examining market milk for the purpose of quickly identifying abnormal samples, inspectors have been limited in their methods to the use of a hydrometer or lactometer, except that in some cases the senses of smell, taste and sight could also be employed to advantage. The determination of the specific gravity of milk has found its chief use in enabling one to select samples which appear to give evidence of being watered or skimmed. It has been repeatedly shown that specific gravity as a basis for accurate judgment in identifying abnormal milks may be wholly misleading. The need of a more comprehensive and reliable method has long been realized.

In making a study of the hydrogen ion concentration of freshly-drawn normal and abnormal milks and also of normal milks subjected to various conditions of change, it was found that the hydrogen ion concentration is very sensitive to certain conditions, among which are (1) the production of acid by bacteria, (2) the addition of formaldehyde, (3) the addition of acids or acid salts, (4) heating above a certain temperature, (5) abnormal or diseased milks, (6) addition of water, (7) addition of alkalis or alkaline salts, (8) removal of fat. The first four conditions increase the hydrogen ion concentration, that is, render the reaction of the milk more acid than normal; while the other conditions reduce the hydrogen ion concentration, that is, render the reaction of the milk less acid than normal.

It occurred to us that if it were possible to obtain an indicator having a neutral point near that of normal milk and yet showing an appreciable color in normal milk, which would be sufficiently sensitive to show observable change of color with slight change of hydrogen ion concentration, such an indicator might find application as the basis of a method to be used for the purpose of quickly indicating the probability of normality or abnormality in a milk. The first suggestion of an indicator meeting these conditions came to us in connection with the work published by Clark and Lubs[1] on "A Substitute for Litmus for Use in Milk Cultures." They made use of a dye, known as dibrom-ortho-cresol-sulfon-phthalein, the name being shortened for convenience to "*brom-cresol purple.*" This dye was found by them to possess properties which make it a reliable and brilliant indicator for the colorimetric determination of hydrogen ion concentration in milk. (This dye can be purchased from Hynson, Westcott and Dunning, Baltimore, Md. In ordering this dye, the full name should be used.)

We first made use of brom-cresol purple in testing its applicability to the detection of increased acidity in milk as developed by bac-

[1]Clark, W. M., and Lubs, H. A. *Jour. Agr. Res.* 10:105. 1917.

terial action, and found that it is extremely sensitive in comparison with phenol-phthalein, which is the indicator in common use in titration for the determination of degree of acidity in milk. Further extension of the use of this dye demonstrated its practicability in detecting other conditions, especially those mentioned above.

Before describing the detailed operation of the method, we will give a brief statement, outlining its main features. The use of brom-cresol purple in this application to the preliminary detection of abnormal milks consists in adding to one drop of a saturated water solution of the dye 3 cc. of milk and then observing the color. In the case of average milks that are normal in character, such, for example, as good market milk, the color is very uniform, being a bluish-gray. In the case of a milk giving a color differing appreciably from this, there is ground for suspicion that it is not normal. The color given by different milks may be lighter or darker, ranging from a bright yellow at one extreme to a deep blue at the other. *The color is made lighter by acids, acid salts, formaldehyde and also by heating above the usual point of pasteurization. The color becomes deeper blue in the case of milk from diseased udders, watered milk, skimmed milk, and milk containing added alkali or alkaline salts.*

If a preliminary test by brom-cresol purple gives a color lighter than normal milk, then the inspector can take a sample to be used in making a further detailed examination in the laboratory for acidity, formaldehyde and overheating. If the color is darker than normal, then a sample is taken to ascertain whether the abnormality is due to addition of water, alkaline salts, removal of fat, or to the presence of milk from diseased udders.

Attention should be called here to certain conditions which modify the characteristic color given by brom-cresol purple with average normal milks: first, the presence of extra fat, as in the case of rich milks; second, the yellow milk produced by pasture grass, and third, the absence or decreased amount of fat as in skim-milk. Milks rich in fat (5 per ct. or more) give an appreciably lighter color than in the case of average milk containing 3 to 4 per ct. of fat, while skim-milk gives a darker color. These differences in color are due to the fact that the fat-globules do not give the reaction color with the dye and they thus modify by their presence the color of the dye in the milk. The fat dissolves some of the dye which always appears yellow in the fat. This effect of milk-fat can be readily observed if one notices the color at once after mixing the milk and dye and then again after the fat-globules have risen to form a cream layer. It will be seen that the color is lighter at the start than it is after the cream has risen and further that the cream layer shows little or no change of color. The difference is more marked with increase of milk-fat.

OPERATION OF METHOD.

1. PREPARATION OF INDICATOR.

Brom-cresol purple is ground to a fine powder and dissolved in distilled water to saturation, about 0.1 gm. being used for 100 cc. of water. Saturation can be hastened by heating the mixture on a water-bath, then cooling to room temperature and filtering. The saturated solution contains about .09 per ct. of the dye.

2. APPARATUS.

The only apparatus required is the following: a burette, test-tubes, pipette and test-tube holder.

The burette is used for the purpose of measuring the indicator. The delivery should be so controlled that each drop measures one-twentieth of one cc.

The tubes which we have found most convenient for use in making the test are flat-bottomed specimen tubes made of pyrex glass, holding about 8 cc.; they are about 4 inches long and ½ inch in diameter. It is essential that all tubes used should be uniform in color and in thickness of walls.

We have found it convenient to provide a special holder for these tubes, making it convenient to compare the color by arranging the tubes in a line side by side in close contact without concealing any portion of the milk column.

Ordinary 3 cc. pipettes are used for measuring the milk to be used.

3. PERFORMING THE OPERATION.

The different steps in carrying out the method consist of (a) measuring indicator, (b) measuring milk, (c) observation of color, (d) interpretation of results.

(*a*) *Measuring indicator.*— The test-tubes are placed in the holder with the open end up. The stop-cock of the burette, filled with the indicator, is so adjusted that it delivers drops measuring one-twentieth cc. at the rate of about one drop in two seconds. The test-tubes are placed under the burette tip one by one in turn, exactly one drop being allowed for each tube. The delivery should be so controlled that each drop falls free from the tip into the test-tube without touching the walls of the tube before the drop separates from the burette. This method enables one to deliver the same amount of indicator into each test-tube with rapidity. There are two advantages in using only one drop of indicator, (1) the dilution of added milk by the dye is inappreciable and (2) the tubes containing one drop can be carried about without danger of losing the reagent.

(*b*) *Measuring milk.*— The milk is added to each test-tube with a 3 cc. pipette and is thoroly mixed with the indicator, which may be conveniently done either by shaking the tube or by drawing the mixture of milk and indicator into the pipette and allowing it to flow back into the test-tube. In our experience the proportion of 3 cc. of milk and one drop of indicator enables one to observe the shades of color to best advantage in most cases, but in some cases we have obtained somewhat better results in observing color changes with 5 cc. of milk for one drop of indicator.

(*c*) *Observation of color change.*— The ability to distinguish shades of color in the change of reaction in milk is the chief point of difficulty in this method and, therefore, the observation of color constitutes the main source of weakness in its application. The method cannot be used successfully by one whose eye is lacking in appreciation of different shades of color to such an extent that training does not enable one to overcome such deficiency. However, in our experience, any person with normal sensitiveness to color changes can acquire the ability to observe such changes as take place in milk treated with brom-cresol purple with an accuracy which will make the application of the method useful. The fundamental difficulty lies in the lack of a fixed color standard which is applicable under all conditions as a basis of comparison. In the examination of market milks, it usually suffices to assume that a large proportion of the samples are normal in reaction and therefore that in a collection of numerous samples of milk those which give the same color with the indicator are generally normal, while those samples which are lighter or darker are open to suspicion of being abnormal in some respect and should be further examined by supplementary methods. It will be well usually, however, especially for those who are unaccustomed to the use of the method, to prepare a series of known standardized colors to be used as a basis of comparison in observing the reaction of unknown milks to which the test is applied.

(1) Preparation of color standard. The prepared color standard represents approximately certain ranges of hydrogen ion concentration. Briefly stated, the preparation of such a standard consists in adding increasing amounts of standard alkali to a mixture of normal milk and brom-cresol purple solution. The preparation of the series of standard colors to be used for comparison is carried out in the following manner.

(1st) Selection of milk. The milk to be used in the preparation of the color standard should meet two requirements. First, it should have approximately the same general composition as that of the milks to be examined; and, second, it should have a normal reaction.

In respect to composition, usually any normal market milk containing between 3 and 4 per ct. of fat will be satisfactory in the inspection of market milks. When milks containing over 4.5 per ct. of fat are to be tested, it is well to use for the standard a milk containing about the same percentage of fat. In the case of skimmed milks, milk with less than the normal percentage of fat should be used. The differences in color caused by the presence of varying percentages of fat can be largely overcome, when necessary, by removing the cream with a centrifuge from all the milks to be examined, using skim-milk in the preparation of the color standard. However, it should be stated that the color given by skim-milk obtained from milk rich in fat may be slightly different from that given by skim-milk obtained from milk poor in fat. After one has had some experience in studying the effects of different milks upon the brom-cresol purple indicator, it will be found that the matter of the selection of milk for making a color standard is simpler than might appear from the foregoing statements.

In respect to the reaction, the milk to be used in preparing the color standard should not have an acidity in excess of the equivalent of 18 cc. of 0.1 N NaOH per 100 cc. of milk; the determination is made by titrating 10 cc. of milk without dilution with the alkali, using 0.5 cc. of a neutralized, saturated, alcoholic solution of phenolphthalein for indicator. The reaction of the milk to be used for the color standard can be further tested by adding brom-cresol purple solution and comparing the resulting color with that given by this indicator with several samples of milk of known normal quality. If the reaction is uniform with that of the known normal milks, the milk can be satisfactorily used for the color standard. Usually, a milk having the acidity equivalent to 1.8 cc. 0.1 N NaOH per 10 cc. of milk is found to be satisfactory.

(2nd) Preparation of standard color series. Measure eight portions of milk of 10 cc. each into separate test-tubes and to each portion add the amount of 0.1 N NaOH indicated below:

Test-tube No.	1	2	3	4	5	6	7	8
No. of drops of 0.1 NaOH	0	2	4	6	8	10	12	14

The alkali is added to the tubes from a burette, using the same precautions in regard to uniform size of drops and their delivery as have been already given for measuring the brom-cresol purple solution. The alkali and milk are thoroly mixed. Of the mixture in each tube take 3 cc. and one drop of brom-cresol purple solution, making a series of eight mixtures contained in the kind of test-tubes previously described. These give a range of colors to be

used as a standard for comparison in testing unknown milks. The reaction color in each tube corresponds approximately to the following pH values:

No. in series...........	1	2	3	4	5	6	7	8
cc. 0.1 N NaOH used...	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
pH value.............	6.5 to 6.6	6.6 to 6.67	6.67 to 6.75	6.75 to 6.82	6.82 to 6.90	6.90 to 6.98	6.98 to 7.05	7.05 to 7.13
Symbol for reaction color	N	N-1	N-2	N-3	N-4	N-5	N-6	N-7

As a matter of convenience in tabulating results, we append a series of symbols to indicate the pH values, N standing for normal reaction, and N followed by the minus sign and figures ranging from 1 to 7 indicating decreased acid reaction corresponding to increasing pH values.

It should be emphasized here that this method gives the reaction only approximately and that the accuracy of the results may be interfered with by various conditions apart from the true reaction. Of such interfering factors, one which is especially influential in affecting the color is the degree of opaqueness. Milks of relatively low acidity tend to be less opaque than those of normal acidity. As a result, the surface color observed is reflected from a greater depth and with a corresponding increase in the intensity of the color in the presence of the brom-cresol purple indicator, thus indicating a reaction less acid than that indicated by the standard. When necessary, this source of interference can be obviated by so diluting the standard series with water after the addition of alkali and before the addition of brom-cresol purple solution that the milk to be used as a standard will resemble in its appearance of opaqueness that of the unknown milk. The milk thus diluted is then used in making up a special standard series by addition of indicator. While the addition of water changes the reaction of the standard somewhat, the test of reaction by the color is made more accurate than if the standard is undiluted.

Another factor that may render less accurate the use of the standard in determining the reaction of unknown milks is the initial reaction of the milk used for the standard. If, for example, the initial reaction is pH 6.5 in one case and pH 6.6 in another, there would be a difference of 0.10 pH in applying the standard to the reaction of unknown milks.

(*d*) *Interpretation of color.*— In examining the samples of milk in the test-tubes after addition of the brom-cresol purple indicator, one makes comparison with the prepared color standard; or, in the absence of such a standard, one selects those samples which differ in color markedly from those samples which according to experienced

observation appear normal, either before or after the separation of the cream. Such samples as appear to be abnormal by showing a deeper blue shade of color, indicating decreased acidity, are open to the suspicion of being watered, or skimmed, or treated with alkali salts, or containing excessive numbers of leucocytes. Which of these suspicions is justified can be ascertained by determination (1) of the freezing-point, (2) of the percentage of milk-fat, (3) of the specific gravity, (4) of the total solids, (5) of the presence of alkaline salts, especially sodium bicarbonate and borax, (6) of the number of leucocytes by direct microscopic examination by Breed's method[2] and (7) of CO_2.

In the case of samples showing a color lighter than normal with the brom-cresol purple solution, indicating an abnormal degree of acidity, there is aroused suspicion of bacterial acid production, the presence of formaldehyde, overheating, the presence of added acid salts, or the lighter color may be due to a high percentage of milk-fat. Which of these indications is correct is determined as follows: (1) A direct count of the number of bacteria by Breed's method is often sufficient. If this fails to show the presence of excessive numbers of bacteria, then a test should be made for the presence of formaldehyde; and, if this is not present, the percentage of milk-fat is determined; and, further, in order to see if the light color is due to overheating, the determination of carbon dioxide[3] should be made and Storch's test may be applied also.

RESULTS OF APPLICATION OF METHOD.

I. RELATION BETWEEN REACTION OF MILK AND THE PRESENCE OF ADDED WATER AND OF GARGET.

We have made application of this preliminary test extensively in the case of market milk and especially in case of watered milks and milks containing garget and also milks with a high bacterial content. Of 570 samples of market milk to which the test was applied, 64 samples, of which 52 showed decreased acidity, were selected by the brom-cresol purple test for more detailed examination in relation to the presence of added water, and high leucocyte content. The results of the work are summarized in Table I. Each sample of market milk represented a single can of milk as delivered at the milk station by the producer. Milk from the same producer was sampled several different days where there was cause for suspecting the milk to be abnormal. Most of the samples giving a normal color reaction (N) were selected from customers whose milk appeared on previous inspection to show signs of being watered, but which gave a normal reaction at the time of this sampling. There are given in addition results in case of a few samples (23–28) of milk

[2] Tech. Bul. No. 49, N. Y. Agr. Exp. Sta.
[3] Tech. Bul. No. 69, N. Y. Agr. Exp. Sta.

obtained from different quarters of udders of cows some of which were known to have udder infection, and also 3 samples of normal mixed milk (6) from our Station herd.

TABLE I.

No. of herd.	Re-action.	Depression of freezing point.	Garget	No. of herd.	Re-action.	Depression of freezing point.	Garget.
		Deg.				*Deg.*	
1	N	0.569	0	13	N-2	0.538	0
2	N	0.558	0	13-a	N-2	0.509	0
3	N	0.558	0	14	N-2	0.477	0
4	N	0.560	0	14-a	N	0.548	0
4-a	N	0.560	0	14-b	N-2	0.530	0
5	N	0.548	0	14-c	N-2	0.512	0
6	N	0.552	0	14-d	N-2	0.490	0
6-a	N	0.555	0	14-e	N-2	0.523	0
6-b	N	0.562	0	14-f	N-2	0.503	0
7	N-1	0.536	present	14-g	N-2	0.513	0
7-a	N	0.546	0	14-h	N-2	0.520	0
7-b	N-1	0.546	present	14-i	N-2	0.525	0
7-c	N-1	0.550	present	14-j	N-2	0.500	0
7-d	N-1	0.536	present	14-k	N-2	0.530	0
7-e	N	0.558	0	15	N-2	0.518	0
7-f	N-1	0.552	present	15-a	N-2	0.536	present
7-g	N-1	0.549	present	15-b	N-2	0.543	present
7-h	N-1	0.549	0	15-c	N-1	0.518	0
7-i	N	0.553	0	16	N-2	0.523	0
8	N-1	0.528	0	16-a	N-2	0.506	0
8-a	N-1	0.547	0	16-b	N-1	0.512	0
8-b	N-2	0.530	0	16-c	N	0.556	0
8-c	N-1	0.545	0	16-d	N-2	0.496	0
8-d	N-1	0.538	0	17	N-2	0.494	0
8-e	N-2	0.541	0	18	N-3	0.519	0
8-f	N-1	0.563	present	19	N-2	0.530	0
8-g	N-2	0.540	0	20	N-1	0.491	0
9	N-2	0.518	0	21	N-1	0.531	0
9-a	N-2	0.531	0	22	N-2	0.540	0
9-b	N-2	0.512	0	22-a	N-2	0.540	0
9-c	N-1	0.530	0	23	N	0.558	0
10	N-2	0.500	0	24	N	0.558	0
10-a	N-1	0.512	0	25	N-4	0.540	present
10-b	N	0.558	0	26	N-4	0.563	present
10-c	N 3	0.470	0	27	N-5	0.558	present
11	N-3	0.468	0	28	N-5	0.548	present
12	N-3	0.519	0				

An analysis of the results contained in Table I leads to the following summarized statements:

1. Of the selected 64 samples of market milk 52 show a color reaction less acid than normal and 12 a normal reaction.

2. In all of the samples giving a normal color reaction, the depression of the freezing-point varies between —0.54° and 0.57°. In the 52 samples of market milk showing a reaction less acid than normal, constituting nearly 10 per ct. of all the samples examined by the color test, 39 samples show a depression of the freezing-point varying from —0.468° to —0.538°, thus indicating the presence of added water. In 18 samples giving N-1 color reaction (pH, 6.6–6.67), 10 samples show watering by the freezing-point, while 8 do not. Of these 8 samples, the decreased acidity is due to the presence of garget. In 30 samples showing N-2 color reaction (pH, 6.67–6.75), 25 contain added water according to the freezing-point, while 5 are just on the border line, showing a freezing-point depression, varying from —0.540° (in 3 cases) to —0.543°. In the case of 4 samples (10-c, 11, 12, 18) giving a color reaction of N-3 (pH, 6.75–6.82), all showed the presence of added water by the freezing-point. In the case of 2 samples (25, 26) having a color reaction of N-4 (pH, 6.82–6.90), and 2 samples (27, 28), a reaction of N-5 (pH, 6.90–6.98), the decreased acidity was due in every case to garget, the depression of the freezing-point showing the milks to be entirely normal in water content. For further details of the relation of the reaction of milk to udder infection, *see* Tech. Bul. No. 70.

3. The number of dairies furnishing the 570 samples of milk examined was 46. In the case of 16, the milks showed a subnormal

TABLE II.

Herd No.	No. of times examined.	No. of samples with subnormal reaction.	No. of samples watered.	No of samples with garget.
7	10	7	0	6
8	8	8	3[1]	1
9	4	4	4	0
10	4	3	3	0
11	1	1	1	0
12	1	1	1	0
13	2	2	2	0
14	12	11	11	0
15	4	4	3[2]	1
16	5	4	4	0
17	1	1	1	0
18	1	1	1	0
19	1	1	1	0
20	1	1	1	0
21	1	1	1	0
22	2	2	0	2

[1] 2 others doubtful.
[2] 1 other doubtful.

or decreased acid reaction by the brom-cresol purple test. Three-fourths of these subnormal samples came from a few herds. It was found that in one of these the milk was being watered regularly and some of the other herds gave evidence of severe mastitis. Table II shows the number of milks of subnormal reaction in the case of the 16 herds, and indicates also the total number of examinations and the number of times the samples were found watered, and in addition the cases where garget was present.

It is seen that in the case of the 16 herds, 58 examinations of milk were made; in 52 samples, the reaction was found to be subnormal or of decreased acidity. In these 52 cases, 37 showed clear evidence of watering by the depression of the freezing-point, and 3 others were so close to the border line as to be open to suspicion of being watered. There were only three herds in which persistent addition of water was shown.

4. The six samples, 23–28, were drawn from the udder under our direct supervision. Four of these show subnormal reaction, owing to the presence of garget; the freezing-point test shows that the percentage of water is not excessive. A complete chemical analysis of these four samples would undoubtedly show abnormal composition according to our work described in Bulletin No. 70 of this Station.

II. RELATION BETWEEN THE REACTION OF MILK AND THE BACTERIAL CONTENT.

Milks showing a reaction above normal acidity, as indicated by giving with brom-cresol purple a lighter color, were examined for their bacterial content. Of the 570 samples examined, 16 gave a lighter color than normal, of which 11 were found by Miss Mildred C. Davis, the city bacteriologist, to contain over ten million bacteria per cc. by the Breed method. In the case of two of the other samples, the light color was found to be due to high milk-fat content, bacteria not being present in large numbers.

A further study was made at the Laboratory of the Department of Public Health of New York City thru the courtesy of the director, Dr. Wm. H. Park. Of the 11 samples found showing light color with brom-cresol purple, 4 contained over one million bacteria per cc. by the plate count and six showed high percentage of milk-fat with low bacterial content.

III. SUGGESTIONS.

It has been stated already that the main source of weakness in the application of this method is the observation of the shade of color given by the sample of milk with brom-cresol purple. It is, therefore, important that before one attempts to use the method in practical application, some special work be done in a study of

the shades of color of the indicator in milk under a great variety of conditions. For example, taking some fresh normal milk of average composition, that is, with 3 to 4 per ct. of milk-fat, a portion is treated with brom-cresol purple in the manner described (pp. 6–7) and then other portions are treated by addition of definite amounts of 0.1 N normal alkali as in preparing the standard series (p. 8) and other portions by 0.1 N lactic acid, while other portions are diluted with definite amounts of water, and others are skimmed, and others have cream added to them. Also the action of definite amounts of formaldehyde added to portions of the milk should be studied, and also varying amounts of sodium bicarbonate, borax, etc. Portions of milk heated to various temperatures are similarly studied. A similar complete study should be made with different samples of normal milk, until one is able to distinguish different shades of color so far as they have a meaning in practical application.

It should be emphasized here again that the application of the brom-cresol purple test is not to be regarded as final but only as preliminary and suggestive. Its chief value is to be found in the fact that, when properly used, it will greatly minimize the work involved in official milk inspection, because it will point in most cases directly to the milks that are abnormal, and therefore indicate which samples need further detailed work to confirm or disprove the suspicion awakened by the result of the preliminary test. We know of no other single reagent which is capable of pointing to so many different conditions.

THE DETERMINATION OF THE KEEPING QUALITY OF MILK.*

J. C. BAKER AND L. L. VAN SLYKE.

SUMMARY.

Brom-cresol purple can be used to measure approximately and relatively the keeping quality of milk. The test is applied in the manner described in Tech. Bul. No. 71 of this Station with the modification that the pipettes and the test-tubes used are sterilized before sampling the milk and, further, the samples of milk in the test-tubes must be incubated a given time at a definite temperature, usually 18° C. to 20° C. The milk is examined for changes in color after certain intervals. The principal factor shown by this test as related to keeping quality is production of acid, but additional factors to be observed in connection with it are coagulation of casein, digestion of casein, production of alkali, production of gas, development of abnormal odor and taste.

In showing the development of acidity, four stages of progress are distinguishable thru change of color, varying from grayish blue of brom-cresol purple in normal milk to a final clear yellow, the intermediate stages showing mixtures of color. In comparing this test with the bacterial count, it is found that in general high numbers of bacteria and increase of acidity are in fair agreement.

The other factors related to keeping quality such as digestion, gas, alkali production, abnormal odor and taste are readily observable, but frequently do not occur until after the first 24-hour period of incubation.

INTRODUCTION.

Keeping quality, or keeping power, is an expression used to indicate the length of time milk remains sweet and otherwise palatable and suitable for direct consumption. This is an important factor in estimating the commercial value of market milk, since milk that is sour or otherwise unpalatable is comparatively valueless as market milk, however rich it may be in fat and other solids.

Various methods have been proposed for measuring the keeping quality or power of milk, such as (1) determination of acidity by titration, (2) estimation of the amount of dirt in suspension, (3) biochemical tests, and (4) bacteriological examination. Such methods, while useful in determining certain factors relating to the cleanliness and sanitary character of milk, have not found a satisfactory application, at least in the forms used, as a means of measuring the keeping power of milk. Titration methods are not sensitive enough to changes

* Reprint of Technical Bulletin No. 72, June, 1919.

of reaction. The amount of dirt in milk is primarily a measure of cleanliness and may or may not bear any relation to the keeping quality of milk. Bacteriological examinations have been used chiefly to determine the cleanliness and sanitary character of milk, but, as shown later, their results are usually of value in measuring the keeping quality of milk, especially in relation to acid production. The technical character of a bacteriological examination limits its use to trained workers. In view of the preceding statements, it is obvious that a simple method is needed which can be relied upon to give consistent results in the approximate determination of the keeping power of milk.

PROPOSED METHOD.

We have found that the brom-cresol purple test (Tech. Bul. No. 71 of this Station) can be applied, with some simple modifications in its technique, to the measurement of certain factors, especially in relation to the reaction, affecting the keeping quality of milk. In applying the test for this purpose, the test-tubes and pipettes must be sterilized before use and the milk in the tubes must be incubated for a stated length of time at a definite temperature. Examination of the milk after incubation furnishes evidence in respect to the keeping quality of the milk as shown by one or more of several possible changes in the milk. Such changes may be divided into two classes: First, those affecting the color of brom-cresol purple solution, showing a change of reaction in the milk due to the production of acid or less often to the formation of alkaline salts; and, second, other accompanying or succeeding changes, such as curdling of the milk due to coagulation of casein, digestion of casein, changes in the physical character of the coagulated or curdled milk, production of gas, and the development of abnormal odor or taste.

In order to be of value as a means of measuring the keeping quality of milk by the reaction, it is essential that the brom-cresol purple under the conditions employed, should not show any germicidal property sufficient to interfere with the growth of bacteria in milk. In order to test this fundamental requirement, pure cultures of *Bacterium lactis acidi* were added to freshly pasteurized skim-milk; one portion of this was treated with brom-cresol purple solution and both portions were incubated at 20° C. (68° F). At intervals the brom-cresol purple test was applied to samples taken from the portion of milk containing no indicator and comparison was made with the portion to which brom-cresol purple had been added at the start. Also samples of the two portions of milk were titrated with alkali. These tests were made repeatedly but in no case was there observable any difference in behavior. The same tests were also applied in numerous cases to two portions of milk undergoing natural souring, using both unheated and pasteurized milk, without showing any observable difference. The results all go to indicate that brom-cresol purple has no germicidal effect under the

conditions used in our work. However, it is advisable to call attention to one precaution in regard to the purity of the brom-cresol purple used in this test, viz.: the dye should be wholly free from the odor of phenol or cresol, as suggested by Clark and Lubs [Clark, W. M., and Lubs, H. A., Jour. of Agr. Research x, 105, 1917.] In our experience we find that a pure product is easily obtained from the manufacturer.

PRODUCTION OF ACID.

In considering the application of the brom-cresol purple test to the measurement of the keeping quality of milk as shown by the formation of acid in milk, we will treat the subject under two heads, (1) localization of acid production and (2) degree of acid production.

1. *Localization of acid production.*— In the natural souring of milk standing undisturbed, acid is rarely developed uniformly thru the body of the milk but is largely localized, especially in the early stages. Acid is usually first formed in appreciable amounts at the upper surface next the cream layer, or less often in the layer at the bottom of the container, or it may appear simultaneously in both the top and bottom layers. In some cases it may start at the side walls of the container. When acid is first formed at the upper surface, it is probably due to the fact that the organisms are enmeshed and carried upward with the rising fat-globules and are thus concentrated in the upper layer. The organisms left in the body of the milk after the rising of the fat-globules would tend, under the downward-pulling effect of gravity, to settle at the bottom of the container. Generally, the number carried up is apparently greater than that carried down. Such a concentration of organisms in the top or bottom layer of the milk would have the effect of making the brom-cresol purple test more sensitive as a result of more rapid formation of acid. The effect of acid development is most commonly shown first in the upper layer, tho sometimes in the lower, or less often in the side layer. But whether it starts at the top or bottom or side, the process of acid production works from the starting area or areas thru the main body of the milk.

2 *Degrees of acidity.*— It would be desirable, if it were possible, to distinguish different degrees of increasing acidity by preparing a color standard representing different values of hydrogen ion concentration, similar to the method described in Tech. Bul. No. 71, for determining the approximate hydrogen ion concentration of milk when its acidity is less than that of normal milk. This is impossible for several reasons and especially because, as pointed out above, the production of acid is localized and not distributed uniformly thru the body of the milk. However, we have found that it is possible, with some experience, to distinguish readily not less than four degrees or stages of acidity by changes of color, varying from the grayish blue observed with normal fresh milk to a pure yellow occurring in milk

sufficiently sour to undergo coagulation (which occurs about pH 4.65). These four stages or degrees of acidity can be distinguished by the following description:

(1) The first stage or beginning of acid production (A_1), is indicated by the first observable change from the grayish-blue color of normal milk to a lighter shade in any portion of the milk. This is most often distinguishable just under the cream layer but may sometimes first appear at the bottom or less often at the side wall of the container.

(2) The second stage (A_2) indicates distinct acid production and is shown when the milk in a test-tube gives evidence of more extensive and marked change than in case of A_1; the main body of the milk, however, still retains a grayish-blue color more or less interspersed with, but predominant over, yellowish or greenish-yellow shades. The prevailing color may be bluish or a dull shade of bluish-green.

(3) The third stage (A_3) shows marked acid production and this is indicated when the color of the milk in the test-tube appears greenish to greenish-yellow; the yellow is predominant, tho not complete thru the body of the milk, but is interspersed more or less with shades intermediate between dull green and yellow.

(4) The fourth stage (A_4) of acid production is easily observable, since the color is a pure, fairly uniform yellow, free from every trace of bluish or greenish tints. The curdling of the milk usually occurs at this stage and is generally, tho not always, readily seen.

The ability to distinguish these four stages of acid production, is comparatively easy with a moderate amount of experience. There will be some cases, when the color may be more or less intermediate between two different stages, but in general the stages are marked with a fair degree of sharpness. With extended experience in color observation, one can differentiate more than these four stages, but in our work these have been found sufficient for practical purposes.

OTHER CHANGES IN MILK.

Changes other than those produced by acid production can also be observed by this method, and to these attention will now be briefly called. These changes may occur only after somewhat prolonged incubation in the case of good market milk, but appear more quickly in the case of milks which have been drawn more than 24 hours before incubation, or in the case of milks drawn under unfavorable conditions as to cleanliness and not kept at a sufficiently low temperature. It should be stated here that while these changes have been studied extensively by bacteriologists, it is essential that they be given special attention and further study under the conditions of the proposed test.

(1) *Production of alkali* during incubation is shown by decreased acidity, which is indicated by increase in depth of the grayish-blue color given by brom-cresol purple with normal milk. We have found cases in which the intensity of blue color increased during

incubation for several days and then remained constant until further changes caused acid production.

(2) *Digestion of casein* is observable, especially just below the cream layer; this is shown by solution of the casein, which at first is just perceptible from the appearance of a slight amount of clear liquid.

(3) *Gas production* is easily observed, indicating the presence of gas-producing organisms. This is especially useful in connection with milk used for cheese-making. There are interesting variations of the appearance produced in the curd by gas, which may have some special significance but which require further study.

(4) The *contraction or shrinking of the coagulated casein* or curd is easily observable when it occurs. This is accompanied by separation of more or less clear whey.

(5) *Any abnormal odor or taste* is readily ascertained by any one having keenly developed senses. Odor or taste, when present after incubation, is generally of an offensive character, and in our experience occurs only in case of milks which show marked change in reaction as indicated by the brom-cresol purple test.

RESULTS OF APPLICATION OF METHOD.

In applying the brom-cresol purple test to the measurement of acid production in relation to the keeping quality of milk, two separate series of experiments will be presented. In the first series the samples used were taken from individual cans of milk as delivered by producers at the collecting stations handling the supply of the city of Geneva. In the second series, the samples were obtained from the regular milk supply of New York city.

In applying the method to 389 samples obtained in Geneva, we have had the cooperation of the city bacteriologist, Miss Mildred Davis, who classified the samples into groups by microscopical examination using the Breed method (Tech. Bul. No. 349, this Station). We are indebted also to Dr. Breed, bacteriologist of the Station, for valuable suggestions in connection with our work.

The results are summarized in the following table:

TABLE I.— COMPARISON OF RESULTS OF BROM-CRESOL PURPLE TEST WITH CLASSIFICATION BY MICROSCOPICAL EXAMINATION.

Class.	Number of individual bacteria per c. c. of milk.	Number of samples examined.	Number of samples changing color.	Number of samples not changing color.	Milk showing good keeping quality.	Milk showing poor keeping quality.
					Per ct.	Per ct.
I	Below 350,000	283	41	242	85.5	14.5
II	Between 350,000 and 1,000,000	21	6	15	71.5	28.5
III	Between 1,000,000 and 10,000,000	52	33	19	36.5	63.5
IV	Over 10,000,000	33	27	6	18.0	82.0

In interpreting the results of the microscopic examination with reference to the fitness or keeping quality of milk for domestic use, milks in class I are regarded as excellent, in class II as satisfactory, in class III as unsatisfactory, and in class IV as very unsatisfactory. While there is a general correspondence between the results obtained by the two methods, the agreement is not complete. In class I, representing milk of excellent quality by the method of direct bacteriological count, 242 samples out of 283 show no change by the brom-cresol test, thus confirming the results of the bacteriological examination; but 41 samples, or 14.5 per ct., of the number examined in this class show sufficient increase of acidity to be detected by brom-cresol purple. In class II, of the 21 samples graded as satisfactory by the method of direct counting of bacteria, 6 samples, or 28.5 per ct., show increase of acidity detectable by brom-cresol purple. In class III, 52 samples are graded as unsatisfactory by direct count, while 19 samples, or 36.5 per ct., fail to show increased acidity. In class IV, 33 samples are graded as very unsatisfactory by direct count, but of these there are 6 which show no increase of acidity. These observed differences of interpretation in the application of the two methods to the determination of the keeping quality of milk are what might be expected under the conditions. The brom-cresol purple test is here applied to detect increase of acidity, while the direct count of organisms includes all kinds and not merely those capable of producing acid. It is obvious that in the case of organisms producing no acid or only very small amounts under the conditions of the test, the brom-cresol purple test would not be expected to apply as it does in the the case of the marked acid producers.

In the case of the work carried on in New York city, the samples were obtained in the regular inspection work of the city milk supply, thru the courtesy of Dr. W. H. Park, Director of the Laboratories of the Department of Health. We are indebted also to Dr. Hazel Hatfield for aid given in the bacteriological work. There were examined 220 samples of unheated milk and 186 of pasteurized milk. The bacteriological counts were made by the official plate method after incubation at 37° C. The tests with brom-cresol purple were made in all cases on samples incubated at 18° C. for 24 hours. This temperature is higher than that found in household refrigerators, but may be regarded as representing the average temperature at which milk is kept after delivery to the consumer.

As will be seen on examining Table II, the milks have been divided into some 15 different classes, a larger number than in the case of the Geneva milks, enabling us to make a more detailed study and show certain relationships more clearly.

The results with unheated milk show a good general agreement. with the brom-cresol purple test. The milks showing large numbers

of organisms show poor keeping quality by the brom-cresol purple test, while those with small numbers show good keeping quality. There is, however, no sharp line of distinction.

TABLE II.— APPLICATION OF BROM-CRESOL PURPLE TEST TO NEW YORK CITY MILKS.

UNHEATED MILK.

Class.	Number of colonies developed per c. c. of milk.	Number of samples examined.	Number of samples changing color.	Number of samples not changing color.	Milk showing good keeping quality.	Milk showing poor keeping quality.
					Per ct.	Per ct.
I	Below 1,000	0	0	0	0	0
II	Between 1,000 and 5,000	4	0	4	100	0
III	" 5,000 " 10,000	6	0	6	100	0
IV	" 10,000 " 20,000	4	3	1	25	75
V	" 20,000 " 30,000	10	5	5	50	50
VI	" 30,000 " 60,000	17	14	3	17.6	82.4
VII	" 60,000 " 100,000	23	22	1	4.3	95.7
VIII	" 100,000 " 150,000	25	20	5	20.0	80.0
IX	" 150,000 " 250,000	57	54	3	5.3	94.7
X	" 250,000 " 400,000	16	14	2	12.5	87.5
XI	" 400,000 " 600,000	16	16	0	0	100.0
XII	" 600,000 " 1,000,000	20	20	0	0	100.0
XIII	" 1,000,000 " 1,500,000	10	9	1	10	90.0
XIV	" 1,500,000 " 3,000,000	7	7	0	0	100.0
XV	Over 3,000,000	5	5	0	0	100.0

TABLE II (*continued*).

PASTEURIZED MILK.

Class.	Number of colonies developed per c. c. of milk.	Number of samples examined.	Number of samples changing color.	Number of samples not changing color.	Milk showing good keeping quality.	Milk showing poor keeping quality.
					Per ct.	Per ct.
I	Below 1,000	4	0	4	100	0
II	Between 1,000 and 5,000	16	1	15	94.0	6
III	" 5,000 " 10,000	9	1	8	89.0	11
IV	" 10,000 " 20,000	32	0	32	100	0
V	" 20,000 " 30,000	32	2	30	94	6
VI	" 30,000 " 60,000	28	3	25	90	10
VII	" 60,000 " 100,000	20	5	15	75	25
VIII	" 100,000 " 150,000	15	9	6	40	60
IX	" 150,000 " 250,000	13	12	1	8	92
X	" 250,000 " 400,000	4	2	2	50	50
XI	" 400,000 " 600,000	5	5	0	0	100
XII	" 600,000 " 1,000,000	3	3	0	0	100
XIII	" 1,000,000 " 1,500,000	5	5	0	0	100
XIV	" 1,500,000 " 3,000,000	0	....	....	......	....
XV	Over 3,000,000	0	....	....	......	....

In the case of pasteurized milks, similar results are shown. However, the milks containing moderately large numbers of organisms show better keeping quality with the brom-cresol purple test than do unheated milks containing the same numbers of organisms.

The results obtained with the New York city milks are not properly comparable with those obtained with the Geneva milks, especially for two reasons. In the first place, the two methods of obtaining the bacterial content, Breeds direct-counting method and the so-called official plate method, do not give results sufficiently comparable for our purpose. In the second place, the New York city milks average probably not less than 24 hours old when the samples are used for laboratory work, while the Geneva samples are not more than 6 to 16 hours old.

In the summary of the work here presented, we do not give the varying degrees of acidity developed on incubation but only the general fact of an increase; more detailed data are being collected and will be presented in a later bulletin.

ADDITIONAL WORK.

While some observations have been made on the relation of the other points to keeping quality, much additional work remains to be done and plans are in hand for further detailed investigations under the conditions of the proposed test along the following lines: (1) Digestion of casein, (2) production of alkali, (3) production of gas, (4) taste and odor, (5) relation of age of milk to temperature and length of time of incubation.

REPORT

OF THE

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REPORT OF THE DEPARTMENT OF ENTOMOLOGY.

CONTROL OF GREEN APPLE APHIS IN BEARING ORCHARDS.*

H. E. HODGKISS.

SUMMARY.

Oviposition by *Aphis pomi* occurs in the autumn, and the eggs hatch the following spring. The maximum numbers of newly-hatched nymphs are ordinarily observed as color is showing in the leaf tips of the opening blossom buds. Development of the insects is rapid, and winged forms of the second generation appear during late May or early June, when there is a migration to other plantings. The species breeds continuously thruout the summer, producing many broods, which vary in size and number according to seasonal conditions.

The green aphis prefers succulent tissues such as exist on terminal growths, water-sprouts and suckers, and is generally present in injurious numbers for more or less extended periods during the summer months in nursery plantings and young apple orchards. In occasional years destructive outbreaks of the insect occur in bearing orchards.

Attacks by the aphis cause curling of apple leaves which may result in defoliation of affected branches. Succulent growth often exhibits a dying back of terminal areas. Invasion of fruit clusters may be attended with dwarfed, misshapen apples which display pimpling and red stippling of the surfaces. The appearance of the fruits is often marred by the sooty fungus (*Fumago vagans* Fries) which thrives upon the excretions of the lice.

Records of the green aphis show destructive outbreaks in bearing orchards during 1903, 1909 and 1912. During June and July, 1918, the species was abundant in many plantings in western New York, and was especially destructive to apple crops in Orleans County.

* Reprint of Bulletin No. 461, June, 1919.

The principal facts and experimental results of an organized effort to control the green aphis during 1918 are as follows:

The green aphis was the dominant species at the time of breaking of the buds. Prior to attack of apple clusters there was generally severe infestation of succulent growth on trunks and large limbs, as water-sprouts, suckers, etc., interplantings of young trees and adjacent newly-planted orchards. Natural enemies of the insects were quite scarce up to the time important injuries became noticeable, and for a period of thirteen weeks there was a progressive increase in the numbers of the aphis. Orchards free from the insects at the time of breaking of the buds showed considerable infestation by midsummer as a result of invasion by winged forms from adjacent plantings.

The delayed dormant treatment protected bearing orchards until about the middle of June, when there was a reinfestation from winged migrants. While plantings that were given the delayed dormant treatment were not conspicuously injured by the aphis, the experiments so far conducted do not indicate conclusively that this treatment may safely be relied on to afford reasonable and satisfactory commercial control.

In the Station experiments a spraying during midsummer resulted in the efficient control of the green aphis. Following the treatment there was noticeable improvement in the conditions of apples in most orchards with respect to shape, size and freedom from reddish discolorations.

Of twenty-three bearing orchards sprayed in coöperation with the Orleans County Farm Bureau, successful results in treatment for the aphis were reported in nineteen plantings. Opinions as to the practicability of the treatment were withheld in three instances, while in one orchard the application was regarded as unprofitable.

Comparative tests of nicotine sulphate with soap or large amounts of lime indicated few differences in insecticidal qualities of these preparations. The advantages of the lime wash were the deterrent action on the aphids and its cleansing properties to the fruits. On account of its lack of surface tension and the difficulty and cost of application to large trees, the use of the lime mixture should properly be limited to young, non-bearing trees or those of moderate size.

The rapid killing with nicotine sulphate in combination with soap and its greater spreading properties point to its superiority for large trees. It is probable, for these considerations, that apple growers having trees of great height with widespread branches will continue to place their dependence on the nicotine sulphate-soap spray for the control of the green aphis.

The conclusions drawn from the Station experiments are that in regions where aphids are annually destructive or attacks are apprehended, reliance should be placed on the delayed dormant treatment with lime-sulphur and nicotine sulphate, and on a supplementary spraying during midsummer with nicotine sulphate and soap when the green aphis threatens to develop to destructive numbers on fruit clusters.

INTRODUCTION.

The green apple aphis (*A. pomi* De Geer) exacts annually a certain toll from New York orchards. It does not usually affect the fruits in old apple plantings, but is most often a dwarfing and deformative agent of the new growth of younger trees. During some seasons the insects breed rapidly and in considerable numbers in older orchards, when they may attack the fruits as well as foliage. In late June, 1918, the numbers of *pomi* in some orchards increased alarmingly, especially in Orleans County. Early in July fruits and foliage were sticky and blackened from the "honey dew" fungus and the growing apples were being distorted. Notwithstanding earlier warnings of danger from the lice, no definite spraying program was being adopted by fruit growers, and in mid-July it became necessary for this Station to direct steps for the protection of apple plantings. Following a survey of this county, special control measures were suggested and parallel tests of various insecticides were made under our direction.

Acknowledgments are due to Mr. L. J. Steele, Manager, for the extensive coöperation of the Orleans County Farm Bureau; to Messrs. Donahue, McCrillis and Mack, Holley; and to Mr. B. G. Wilson, Carlton, for the use of their orchards for experimental purposes and for their personal assistance, which aided greatly in the successful prosecution of the work.

HISTORY OF THE SPECIES IN BEARING ORCHARDS IN NEW YORK.

The early records of *pomi* appear to be confused to a considerable extent with those of *avenæ*. Accounts of unusual outbreaks, however, have come to us with much accuracy, despite a paucity of early knowledge of the separation characters. Our more definite records coincide in point of time with the renewed interest in fruit growing and the development of more modern methods of spraying. Such reports bear evidence that in all these years the green apple aphis has been of more or less casual abundance, and only at long intervals of time has it been of importance in bearing orchards. The first severe infestation of the past twenty-five years was in 1903. Prof. M. V. Slingerland in writing of this outbreak mentioned that "one orchardist thinks his apple crop was reduced one-half by the lice, and a prominent buyer reported it was difficult to pack good stock in the infested orchards."[1] As to the damage to nursery plantings, he stated that two leading nurserymen estimated their loss at $5000.00 each. The following year (1904) saw a return to normal conditions, which was succeeded by several seasons when the aphids were not a factor in the production of apples. The summer of 1909 experienced another onslaught which was comparable to the outbreaks in 1897 and 1903. The literature for 1909 does not state clearly the extent of injury caused by *pomi*, but from unpublished records it is evident that the species was responsible for considerable damage to young trees. The insect did not attract serious attention again until 1912, when in June of that year considerable apprehension was caused by the abundance of the aphids, especially in plantings of younger trees and on nursery stocks. In bearing orchards the inroads of the lice were accompanied by serious defoliation of the trees and extensive injuries to the apple crop. For the next two summers *pomi* existed apparently in unimportant numbers. During June, 1915, young non-bearing trees were severely injured by this species, and on June 22 at Geneva it was necessary for owners of nurseries to resort to spraying in order to prevent further injuries. Older bearing trees were not immune from attacks, but at no time were the lice a serious

[1] Slingerland, M. V. Proc. W. N. Y. Hort. Soc., 49: 72, 1904. Rpt. Bu. Farm. Inst. N. Y., pp. 179–184, 1903 (1904).

menace to the young fruits. During succeeding years *pomi* was again unimportant until in 1918 there occurred a destructive outbreak which was comparable to that of 1903.

LIFE HISTORY AND APPEARANCE OF THE INSECT.

Egg.— The egg is oval in form, and is somewhat flattened on the side next to the bark. It does not differ from *Aphis avenæ* in shape, but is, on the average, slightly longer. The smallest eggs of *pomi* are, however, shorter than the longest ones of *avenæ*. The width in both cases is the same. Ova of *pomi*, in our studies, averaged 0.62 mm. in length and 0.26 mm. in width. The extreme measurements were 0.59 mm. to 0.66 mm. long by 0.25 mm. to 0.28 mm. wide.

When deposited, the egg is pale greenish-yellow. In a short time it becomes dull, dark green, and finally changes to a glossy black. On young apple stocks in the Station nursery during the autumn of 1914 permanent coloration of *pomi* eggs became fixed in four days. During the following season the color changes occupied five days.[2]

Deposition takes place in the fall. The eggs may be laid singly or in rows in the creases at the bases of the buds, or in collections in scars on the young bark. They are rarely seen on the larger limbs or on the trunks of older trees. It is common to find them scattered over the more succulent stems and young wood, and sometimes the bark is almost obscured by the minute ova, especially wherever the aphids have been abundant. The species hibernates only in the egg stage.

Stem-mother.— This is the name given to the nymph of the first generation, which is the only brood developing from a sexual egg. At hatching the young nymphs are not unlike first-stage individuals of *Aphis avenæ* and *A. sorbi* in gross anatomy. A close examination of the initial stages discloses certain differences in color and external structures which provide easy identification characters. Newly-hatched nymphs of *pomi* are most often of a dark, dull green color, while the corresponding stages of *sorbi* and *avenæ* are somewhat darker. The essential features of the first-stage nymphs are

[2] Baker and Turner (*Jour. Agr. Research*, 5: 960, 1916) give this period, under artificial conditions, as "one to four days, usually a little over one."

the size of the antennæ and the button-like "honey tubes" which are intermediate between those of *avenæ* and *sorbi*.

Summer form.— As maturity approaches, the stem-mother becomes larger, and is distinctly unlike either *sorbi* or *avenæ*. Adults of *pomi* are plain green in color and, except for individuals of a dull yellowish tinge, the stem-mothers are of a uniform green with black appendages. The head and thorax usually have a whitish powdery coating. The adult is wingless, and produces both winged and wingless offspring. Several generations of similar forms occur during the summer. These are composed mostly of wingless females. Winged forms do occur in each later brood, but they are not as abundant as in the second generation.

The sexes.— In early autumn, wingless sexed individuals are produced. These are of a pale color. Within a short time after mating, the females deposit eggs which, according to Baker and Turner,[3] must develop to a resting stage before the first heavy frosts; otherwise they may be winter-killed.

SEASONAL ACTIVITIES OF THE APHIS.

The eggs of *pomi* are deposited during late October or early November. The period of oviposition at Geneva during 1914 was nineteen days. That year eggs were being deposited in considerable numbers on October 23. Ordinarily the first eggs of *pomi* have been observed between November 4 and November 8. Hatching of the eggs occurs with the approach of warm weather in the spring. In western New York the first appearance of young nymphs is usually coincident with the appearance of color in the leaf tips of the blossom buds. At this Station a record of the hatching of *pomi* during a period of ten years indicates that, in the main, there is but a slight variation in the first appearance of nymphs of this species in the spring. These records are given in Table I.

Stem-mothers of *pomi* usually mature as color is showing in the blossom clusters. Seasonal conditions apparently produce some variation in the time consumed between the hatching of the eggs and the appearance of the adults, as is indicated by the records for the years 1915 to 1918. During this period adults of the earliest nymphs of the first generation at Geneva were noted on the follow-

[3] Baker and Turner. *Jour. Agr. Research*, 5 : 955, 1916.

ing dates: 1915, May 4; 1916, May 13; 1917, May 26; 1918, May 10. During these years pink was abundant in apple clusters May 6, 1915; May 17, 1916; May 27, 1917; May 9, 1918.

TABLE I.— RECORD OF HATCHING BETWEEN 1909–1918.

Year.	Date of hatching.	Year.	Date of hatching.
1909	April 23	1914	April 24
1910	April 3	1915	April 16
1911	April 22	1916	April 26
1912	April 20	1917	April 22
1913	April 14	1918	April 20

In 1917, nymphs of the second generation were first observed during the period of May 26–28. The following year (1918), development of the lice was much earlier, which was indicated by the finding of the second generation larvæ on May 12, and reproduction thereafter was very rapid. On June 11, 1917, some individuals of the second generation were developing wings. Ten days afterwards a distinct flight of winged adults took place, which was coincident in point of time with the migration of *pomi* in 1915 and three weeks later than that of 1918 which occurred on May 31.

A few nymphs of the third generation appeared on June 9, 1917. As did the preceding broods, this one developed much earlier during 1918. In outside breeding cages, a number of newly hatched nymphs of the third generation were detected on May 19. In orchards, the progeny of winged migrants were not numerous until June 9. Subsequently the lice increased rapidly until about July 22 when, owing to the transformations of the insects and reductions in numbers by parasites and predaceous enemies, they apparently became less abundant.

In the vicinity of Geneva sexual forms develop usually in the month of October. During the period of 1912–1918 the first specimens were observed between October 13 and October 21. Two notable exceptions were the years 1912 and 1917, when no sexed forms appeared on the trees until November 8 and November 4 respectively. In some years winged fall migrants have been noticed

quite late in the autumn. In 1912 apple foliage remained on the trees until early in December, and on December 6 two winged forms were found accompanied by several young sexed individuals. At this date, however, the sexes were mostly mature; and on December 14 a note was made that all were adult, and less than one-third as many were on the apples as observed on December 6.

ACTIVITIES OF THE INSECTS DURING 1918.

Situation in the commercial apple regions.— The area affected by the unusual abundance of *pomi* during 1918 embraced the entire fruit-growing section of western New York. In some districts attacks of the lice were of local importance, while in other centers heavy losses were averted only by timely spraying. The area most seriously threatened by the outbreak was in the eastern portion of Orleans County. In other counties the damage to the apple crop was apparently not of great importance, and certainly not of such serious extent as was feared at the beginning of the onslaught.

It is interesting to note that, on June 7, exceptional activities of the insect were observed in the territory bordering on Lake Ontario, especially in the counties of Niagara and Orleans. As the season advanced, orchard conditions in Niagara County improved somewhat. Treatments in restricted areas would undoubtedly have effected a better size of the apples on the average, but the Farm Bureau Spraying Service considered a general campaign against the pests inadvisable owing to the slow rate of increase of the insects and the comparative freedom of the fruit clusters from infestation. Similar conditions prevailed in Monroe and Wayne counties and, while it became necessary to combat *pomi* in some orchards, spraying was in the main restricted to individual plantings.

In Ontario County the lice early sought the water-sprouts and succulent terminals of bearing trees. Attacks upon the young apples were not general, the most conspicuous evidences of the work of the pest being seen in badly discolored foliage and fruits due to the " honey dew " exuded by the insects, and its attendant fungus.

About Oswego and other apple centers to the extreme east of the western apple belt few instances of damages by the lice were noted; and it appears that these were mostly in orchards where, ordinarily,

a high degree of orchard cultivation is practised, with the attendant advantage, from the insect standpoint, of the existence of considerable amounts of succulent stems common to trees in a vigorous growing condition.

The apple section of the Hudson Valley furnished fewer complaints of the green apple aphis than the Lake region. From reports of county agents, however, it appears that the orchard conditions as regards this insect were quite similar to those of western New York outside of Orleans County.

Early spring conditions in Orleans County.— The spring migration of *pomi*, which occurred during the last week of May, 1918, preceded by a few days an inspection of experimental plats in some of the larger bearing apple orchards in the vicinity of Albion and Medina. During these explorations it was noticed that winged migrants were scattered throughout the plantings. In some instances the winged forms were so numerous that the attention of officers of the Farm Bureau was called to the situation, and a warning was given, coupled with the suggestion that a close watch be kept on the lice, as conditions might render spraying advisable. During a subsequent visit to this section on June 18, it was observed that *pomi* nymphs had stunted the terminal growths of apple trees, and the leaves, even of fruit clusters, were being curled. In certain instances, the insects were seen on apple stems and young apples.

The most severely infested orchard at this date was the extensive Baldwin planting of Mr. Clark Allis, Medina. These trees, which are of unusual height, and have widespread branches, were so badly overrun by the lice that terminals, fruit and leaf clusters, and watersprouts were deformed and curled. The foliage was smeared and dripping with "honey dew" and the apples were sticky. Aphids were feeding on the fruits, and many of the little apples were coated with the pests. Young apples that were attacked showed some roughening or pimpling at the calyces. On some of the larger fruits a reddish stippling occurred. The situation in this planting was so serious that an immediate application of an aphidicide was recommended, especially to the lower portions of the trees which at that time were overrun with the insects and were sustaining the most severe injuries.

In other plantings of standard varieties of apples, the multiplication of *pomi* was not as rapid. The general situation, however,

appeared to be less satisfactory each day and, as it was likely to become serious, a note of warning was again sounded through the Farm Bureau advising apple growers to watch the trees, and, if the young fruits were attacked, to start spraying. Conditions later in the month seem to be more favorable for the trees and only local attacks were recorded; when suddenly, about the middle of July, the onslaught became intensified, and several growers in the vicinity of Holley requested immediate assistance as the green aphids were said to be causing serious losses in their orchards and neighboring plantings.

Midsummer conditions.— In response to these demands, the Station assumed the responsibility of advising growers as to the most efficient spraying practices. In order to meet more fully the situation, it was necessary to inaugurate a survey of the principal apple areas to ascertain to what extent the renewed attack was causing damages.

Beginning at the county line road west of Lyndonville and including the territory between the railroad and Lake Ontario eastward to the Monroe County line, the lice were breeding abundantly causing serious injuries in orchards not already sprayed and inflicting damage to a somewhat less extent in sprayed trees. Plantings of old and younger bearing trees were similarly attacked. The younger plantings of apples appeared to be more severely injured than the older trees, which was undoubtedly due to the greater susceptibility of succulent growth to the dwarfing influence of the insects.

The attacks of *pomi* in orchards in the section north of the Ridge road and extending to the railroad appeared to be only of individual significance.

In the section around Medina, Knowlesville and Albion, most of the larger plantings were being damaged by the lice. The infestation was not equally extensive or destructive in all orchards. In some of them single trees or parts of trees were attacked, and these injuries alone were sufficient to cause important reductions in the yields of apples. In such instances applications to the infested portions of each tree often secured the necessary relief. In a number of instances, especially where several varieties were interplanted, the blocks comprising early maturing kinds were likely to be more infested than later ripening varieties in adjoining plats. It was not

uncommon to find individual trees, or parts of trees, severely harmed by the lice, and the balance of the orchard with either no infestation or with a few areas only subject to attacks.

In the region to the east of Albion, and especially in the vicinity of Holley and Fancher, the conditions were apparently most favorable for the rapid development of the insects. In this section nymphs of *pomi* were so abundant during July that it appeared as if the pests were forced to go to the fruits as well as to the less tender foliage in an endeavor to sustain life. On July 18 the fruit clusters, terminals and young fruits were being blackened and deformed thru the activities of the miscreants, and the newer terminal growths were stunted.

In this section, cultivated orchards were badly injured. Neglected plantings or those subjected to less advanced orchard practices were ordinarily little harmed by the pests. Trees that were growing closely together and had interlacing branches with luxuriant foliage were more apt to harbor numbers of the insects than trees of the same age and size but with better air drainage. Plantings of comparatively young, bearing apples were in many instances less affected than larger trees of the same variety. The most notable injuries occurred in the larger bearing orchards where growth was luxuriant, and where the low-hanging branches prevented good air circulation. Coupled with this dense foliage was an abundance of water-sprouts, which are always centers of infestation even in normal seasons. Closely planted trees were invariably breeding centers for extensive numbers of the lice. One of the orchards visited serves to illustrate how the character of planting and succulent foliage present unusually favorable breeding areas for the insects. The trees here were originally set too close, and, eventually growing together, it became necessary to remove the alternate ones thruout the orchard. When the work was undertaken, the trunks were denuded of limbs and branches, and left to be removed as opportunity offered. During the past year these stumps, on account of the abundance of stems and water-sprouts, had again become sizable trees, in many instances occupying a large part of the space taken by the original trees. The woody growth was composed of medium sized succulent stems and older suckers. Such growths were found to be heavily infested with *pomi*, and it was believed that these were centers of infestation from which the lice had spread

to adjacent trees. The timely removal of the stumpy growths aided considerably as a supplementary measure in the work of suppressing the insects.

The apples most affected were Twenty Ounce, King, Duchess, Greening and Baldwin. No variety was immune from attack.

It was an interesting fact that there was a noticeable absence of predaceous and parasitic enemies of *pomi* thruout the entire region. In this respect the conditions were quite similar to those in the neighboring counties. Predaceous enemies and parasites did not increase in numbers until toward the end of July, when maggots of syrphid flies and parasitized *pomi* nymphs became abundant. They did not exert a marked deterrent effect on the lice until about July 22, when practically all the injuries to apples had been accomplished. At this time the force of the invasion was spent, and in orchards where no attention had been paid to the work of the lice little benefit accrued from the activities of these agents.

CHARACTERISTICS OF THE OUTBREAK.

Effects on the foliage.— The newly hatched nymphs of *pomi*, as is the case with other aphids, were attracted to the tender leaves of opening fruit buds or the more advanced terminal buds on non-bearing trees. As growth advanced, the lice continued to feed on the newer unfolding portions in preference to the older foliage. This feeding habit has, in ordinary seasons, often led casual observers to state that the species is an unimportant factor in the curling of the leaves. Nevertheless, it is evident that, even in those seasons, curling factors largely in the growth of young trees, and may result in serious injuries if, as during the past year, the aphids breed without restriction of their numbers by repressive agents. The colonization of the insects had a noticeable influence on the character of the injuries. During feeding, if only a few lice were on the leaves, little harm to them could be detected, while, in instances of a greater abundance of the pest, curling of the leaves was for the most part quite severe. The condition of early fall apples in the Station orchard was typical of the first attacks of the insects. Here the water-sprouts, attacked by colonies of *pomi*, were on May 25 badly curled and twisted, much like the characteristic work of *sorbi*. On younger trees and in nursery plantings, the leaves were incurved

and blackened, and the terminals became stunted. Where infestations were unusually severe, bushy growths often resulted. The curling was proportionate to the infestation, and varied from a slight incurving of the leaf margins to a pronounced curl, and in some instances a twisted effect was produced.

Bearing Greenings of moderate age became stunted, and the leaves yellowed and dropped. On older trees, the discoloration in most instances was not so evident. Terminal growths and leaf clusters showed marked evidence of dwarfing effects, however, and the development of fruit buds was considerably retarded. Late in the summer, buds on unsprayed trees lacked the plumpness of fully matured ones on uninfested clusters. Some trees exhibited a different type of injury which was indicated by a shriveling and stoppage of growth on the woody stems.

The attack was accompanied by the usual smearing of foliage with "honey dew." Scarcely an orchard was seen where these secretions were not dripping from the leaves, and even covering the ground beneath the trees. Accompanying the sticky condition the "honey dew" fungus was abundant. On trees of all ages this material coated both surfaces of the leaves. Leaf petioles, axils of the stems, buds and woody growths became blackened, which contributed to the generally unhealthy appearance of the foliage. The leaves also assumed a peculiar dark bluish-green color. This appearance was quite characteristic of infested plantings, and, even after the aphids became reduced in numbers, the foliage continued to be abnormal in color.

Feeding activities on fruits.— Early attacks by *pomi* on young stems and newly formed apples resulted in a stoppage of growth, and a dwarfing of the fruits. Accompanying the injury was a shortening of the axial diameter which produced a type of injury characteristic of this species. Around the calyx cavity the fruit was pimpled or the surface raised to form warts. Fruits attacked when quite small were usually dwarfed, and hung to the stems as "cluster apples." The stems became fixed, and affected fruits were often removed with difficulty. When the fruits had attained some size before infestation, the calyx was either flattened or pointed, humped or laterally distorted, depending on the variety of apple. Some apples exhibited a transverse flattening, usually on one side, while the opposite sector was always more plump than round. This

condition was more often found where the lice were protected at the points of contact of apples in a cluster or where leaves rested on the fruits. In some cases the surface was pitted or irregular and rough to the touch.

When feeding was of comparatively short duration, external malformations of the surface were not common, altho a dwarfing tendency was evident. In some instances red spots or stippling occurred on the skin of young apples, giving an appearance similar to that of fruits infested with San José scale. All the affected fruits were not stippled, but from the apples examined there was sufficient evidence to indicate that *pomi* is at least a causal agent in this particular blemish. It appeared that these fruits had been attacked at a period in their growth when the tissues were most susceptible to the reaction of some toxic material secreted at the time the surface was punctured by the aphids. The discoloration was external only, which differentiates this stippling from the spotting by the San José scale. (Plate XXI.)

The pulp of *pomi* apples was of a mealy texture. In such fruits there occurred a distinct watery area just beneath the skin. In a bisected apple it appeared as a narrow marginal line, resembling in some respects the affection known as "water core." This margin was irregular in outline and in width. It almost disappeared in some instances, and seldom extended beyond the outer ring of the fibro-vascular bundles. Similar conditions have been found in apples injured by the rosy aphis.

The arrangement of the carpels, or seed cavities, was noticeably distorted. In cross sections of apples the abnormal structures were found on comparable sides of the fruits. Such carpels were seedless, or the seeds were deficient in strength, as was indicated by their size. Seeds, where present, were much smaller than those of normal fruits.

Injuries which resulted in the dwarfing or misshaping of apples occurred largely between June 15 and July 20. Rome apples infested on July 22 were undersized and blackened, but exhibited none of the curious shapes which accompanied the feeding earlier in the season. On August 1 twelve clusters of Rome apples were infested with a number of colonies of *pomi*. Each cluster, which comprised two or three fruits, was enclosed in a net bag, insuring a continuous infestation of the apples. These were picked on October 21, at

which time the fruits were coated with " honey dew " and blackened, but it was not apparent that they were on the average smaller than apples which had not been attacked by aphids. The interior structures of all the apples in the test were normal in every respect.

STUDIES ON CONTROL MEASURES.

PLAT EXPERIMENTS.

THE DELAYED DORMANT SPRAY AS A PROTECTIVE TREATMENT.

In our previous bulletins on apple aphids, attention has largely been directed to the benefits derived from the delayed dormant applications in protecting trees from newly hatched lice and their progeny. The migratory activities of the green apple aphis counteract in a measure the benefits from the early spraying as regards this species, altho it is believed that the bud spray exerts a strong repressive action on the multiplication of the insects. That the protection afforded cannot be relied upon to extend beyond the period indicated is shown by the June migration of adults. In view of the fewer cases of extreme infestation in orchards where the early application with nicotine sulphate was thoroly made, attention is directed to conditions which seem to indicate that the delayed dormant spray was in some circumstances a deterrent to the activities of the lice.

Five or more experimental plats in the vicinity of Albion are being sprayed each year to demonstrate rosy aphis control, using lime-sulphur and nicotine sulphate in the delayed dormant application. A slight infestation of *pomi* occurred in most of the tests. In three orchards only an occasional leaf or fruit cluster was infested. Foliage on terminal growths and water-sprouts was not badly injured and the leaves appeared normal in every respect. In one orchard neither sprayed nor check trees were infested. The fifth plat was a planting in which the only spraying made was to the trees in the experiment. In mid-June the treated trees were free from any infestation, but water-sprouts and terminals of the check trees were being quite badly injured by the pests. On July 18 the lice were being distributed over the experimental trees in sufficient numbers to make a supplementary treatment of the plat advisable. The balance of the orchard was severely injured by this time, but despite the reinfestation of the test rows, the condition of the checks stood out

strongly in contrast to that of the trees receiving the delayed dormant spraying. It is interesting to record in this connection the experience of an orchardist in the town of Kent. His planting is sprayed for aphids each spring, and ordinarily the chief sources of infestation are nearby orchards. In the rush of spraying in a block of 40-year-old Greening apples, three trees were inadvertently left untreated. On June 18 the leaves on these trees were gummy and badly curled, and the young wood was blackened. The lice were so numerous on the fruits that instances were rare where an apple was not entirely coated with the pests. The surrounding trees were consequently rather heavily infested. The attack, however, lessened in intensity as the distance from the center of infestation increased, and while some lice were scattered through the orchard the attack other than mentioned was not large.

Winged migrants were detected in the Station Rome orchard on May 31. A portion of the planting received a protective spraying, but it was observed at the time of picking the crop that the check trees were quite sooty, and gum still adhered to the apples; while the trees receiving only the bud spray were comparatively clean.

TESTS OF SUPPLEMENTARY APPLICATIONS.

Despite the favorable impressions conveyed by our observations in orchards receiving the delayed dormant spray, experience has indicated that the usual contact remedies, while efficient aphidicides, have a limited range of effectiveness when used against the green apple aphis, owing to its migratory habits. To counteract the cumulative injuries by this aphis and to provide some protection in orchards not sprayed early in the spring, it became necessary to find an aphidicide which would combine the insecticidal properties of the contact spray with some irritant preparation not harmful to the plant but objectionable to the insects. During the summer of 1914 a heavy lime wash in combination with nicotine sulphate was used with good results against the pear psylla. Believing that this combination would be of similar advantage in protecting apple leaves during a prolonged attack of *pomi*, experiments were conducted in the Station plantings during 1915 to test its merits.

Experiment 1.— The trees selected for the experiment were seedlings of standard varieties about five years of age, and, without exception, the foliage was luxuriant. On June 22, 1915, the terminal

growths were generally infested with adults and nymphs of *pomi*. As the lice had not become abundant, very few injuries had been effected. In this test 807 trees were sprayed or left as checks. The sprays applied were nicotine sulphate either with soap or in a heavy lime mixture according to the following formulae:

Formula 1. Nicotine sulphate-Soap.

Nicotine sulphate (Black Leaf 40)	3/4 pint
Fish-oil soap	4 pounds
Water to make	100 gallons

Formula 2. Nicotine sulphate-Lime.

Lump lime	60 pounds
Copper sulphate	4 pounds
Nicotine sulphate (Black Leaf 40)	3/4 pint
Water to make	100 gallons

The weather during these tests was generally fair. A heavy rain followed the completion of the first day's work, but subsequently conditions improved and continued favorable for some days after the treatments were made.

The several tests were:

Plat I. Preparations of the foregoing mixtures were applied to a total of 264 trees. Following a rain at night, 67 of these were resprayed with the nicotine sulphate-lime wash.

Plat II. The sprays were repeated on 533 trees in adjoining rows.

Plat III. On July 20 a block of 270 trees was resprayed, using the heavy lime mixture as before, the mixture being prepared according to the same formula except that the slaked lime was washed before mixing. This washing process consisted of allowing the slaked material to settle, after which the water was run off and fresh water added in the barrel. The washing was repeated twice before the material was applied. Some of the trees had received the earlier treatment with nicotine sulphate and soap.

Plat IV. A treatment with the nicotine sulphate-lime combination was repeated on 48 trees August 2. The purpose of this test was to ascertain if additional immunity could be obtained from aphids by a third application of the lime wash.

Results of the experiment.— Plat I. The two sprays differed little in effectiveness. The nicotine sulphate-soap spray was immediately effective, and nearly all the insects were destroyed. The only lice

not harmed were some scattering colonies so protected by the leaves that they were not wetted by the spray. The nicotine sulphate-lime wash was somewhat slower in action, but on the next day only scattering nymphs were alive, and these apparently were being bothered by the heavy coating on the leaves. The heavy rain immediately following this spraying washed the trees somewhat, which lessened the protection from subsequent broods of the lice. Counts of curled leaves were made three weeks later, and on trees sprayed before the rain 30 per ct. of the leaves were curled; while on those treated after the precipitation the amount of leaf-curl was reduced to 6.7 per ct.

Plat II. The difference between Plat II and Plat I consisted in the coating of the leaves with lime. The heavy coating continued to act as a deterrent until considerable new terminal growth developed. As the lice migrated from adjacent untreated trees to these terminals, the protection was in a measure lost. When compared with the nicotine sulphate-soap sprayed lot the benefit of the lime was apparent, as the soapy spray in no wise prevented a reinfestation of the leaves, lice continuing to cause injuries to the trees during the summer. The leaves coated with lime were, however, free from the lice, and exhibited no tendency to curl. There was also a noticeable lack of "honey dew" and the accompanying blackening from the fungus was absent. The average amount of curled leaves on this plat, which received one application, was 7.5 per ct. for the lime-sprayed trees and 24.2 per ct. for the nicotine sulphate-soap combination. The trees receiving the latter treatment were not very different from the checks as regards the extent of malformed foliage.

Plat III. These trees received a second spraying with the heavy lime-wash, which added considerably to the amount of protection from the lice. In September a count of affected foliage on a number of trees indicated 3 per ct. of curled leaves on the trees sprayed twice as compared with 7.2 per ct. on those sprayed once. The checks in this plat averaged 22.1 per ct. distorted foliage. The application of the nicotine sulphate-lime following the earlier one with nicotine sulphate-soap was quite beneficial and, aside from the original curling, these rows appeared as well as the trees which had received the two lime sprayings. Washing of the lime resulted in almost entire freedom from the tip-burn, which was not uncommon

on the newer leaves of trees treated with the unwashed preparation. There was, however, an individual variation in this respect. The advantage from the washing was not, as a whole, considered sufficient to pay for the additional labor involved.

Plat IV. The third treatment with the lime combination killed all the lice hit by the spray and, as an additional protection, proved advantageous, altho the force of the outbreak was spent, and the season's growth was already quite woody. This application in some seasons would undoubtedly be of value, especially on young trees where the attack was unduly prolonged.

Experiment II. Six standard bearing apples were sprayed on July 10, 1915, using the nicotine sulphate-lime formula. One-half of each tree was treated, while the other section served as a check. A second application was made on August 2. All the trees were carrying quite a crop of fruit, and it was interesting to note the differences in the conditions of the sprayed apples and the checks. The unsprayed portions of the trees were considerably blackened, and the leaves were sticky. The apples caught some of the dripping "honey dew," and were likewise discolored. On the other half each tree bore fruit that was clean and of a high quality. The only sediment was some that was forced into the calyces at the time of spraying, and their later inverted positions prevented the rain from removing the particles of lime which continued to adhere in small amounts to the blossom ends of the apples.

Experiment III. On July 10, 1915, six apples of standard varieties on dwarf stocks were sprayed with the nicotine sulphate-lime wash. The trees were about 17 years of age, and that year bore a good crop of apples. The test was to ascertain the effects of the spray on the leaves and fruits as well as on the aphids. To provide a check, one-half of each tree was untreated. On August 2 the treated half of each tree was resprayed. In these tests the leaves, especially the terminals, were protected by the application. Curling of the leaves was unimportant, and none were injured after the spray was applied. The coating on leaves and fruit wore off to some extent, but it continued to function until the time of treatment in August. The respraying added appreciably to the protection of the foliage, and after the spraying the lice were not a factor in this planting. The effect of the lime on the fruit was not injurious, and at picking the only traces of the coating were a few granules in the calyx

cavities or at the stem ends of the apples. Whatever deposit remained in no wise affected the marketable value of the fruit.

EFFECT OF LIME ON QUALITY OF APPLES.

Aside from the protective value of the nicotine sulphate-lime spray, some hesitancy may be felt in using the material on bearing apples, due largely to the question of its adhesiveness to the fruits. To provide data on the conditions of treated apples at harvesting, sprayings were made with this wash in comparison with the nicotine sulphate-soap combination. The tests were made during 1916 and 1917 in the Station apple plantings as follows:

Test I. On June 1 and 2, 1916, forty trees about thirty years old were sprayed, using nicotine sulphate with either lime or soap according to the foregoing formulae. In the nicotine sulphate-lime spray two gallons of lime-sulphur solution replaced the copper sulphate as the adhesive. Portions of these trees were resprayed on June 22, using only the heavy lime combination.

Test II. Sixteen Rome apples about twenty years old were sprayed on July 10, 1917, with either lime-sulphur and nicotine sulphate in the usual proportions or the nicotine sulphate-lime wash. Arsenate of lead was added to each mixture for late larvæ of the codling moth.

In both tests *pomi* was not a serious factor. During each year enough lice were breeding on terminal shoots, leaf clusters and water-sprouts to cause curling of leaves and smearing with "honey dew." The fruit on unsprayed trees was usually normal in size, but became more or less streaked and discolored as a result of "honey dew" dropping from the leaves.

In Test I the trees had an abundance of apples. The fruit and foliage were thickly coated with lime as a direct result of the application. The trees sprayed twice had a proportionately heavier coating thruout the summer than those treated earlier. It appeared that, where applications were made as early as June 1 and 2, the covering did not give sufficient protection to either leaves or fruit, altho it provided a film well into August. Lime from the second spraying remained on the fruit thruout the season. The coating, which was heavy during July, gradually disappeared, and at harvesting only that which had collected in the blossom end remained on most apples. On the variety Greening a film of lime

usually remained on the skin, altho this was largely removed by handling at harvest. The lime on these apples in no way affected their marketable value, and some of these fruits were used for exhibition purposes without being cleaned.

Test II was conducted in the Station planting of Rome apples. The attack of *pomi* was of small proportions, altho the check apples were all more or less discolored from the effects of "honey dew" dripping from the upper centers of infestation. The fruit on the rows receiving lime-sulphur and nicotine was freer from aphids than the checks. The attendant blackening from "honey dew" remained, with the result that, at harvesting, these apples were not much different from the checks. Those treated with lime, however, had an entirely different appearance. The surfaces were clean and of normal coloration, and in October only a few particles of the lime remained. The contrast was quite striking since, when the fruit was sorted, all but a few lime-treated apples were classed as Grade A. When the black, sticky fruit on both the checks and trees receiving lime-sulphur and nicotine was rejected, it was estimated that sufficient loss had been sustained to have paid for spraying the balance of the orchard with the nicotine-lime wash.

LIME AS A DETERRENT TO POMI ATTACKS.

Test No. I. During the first week in June, 1918, breeding of the lice indicated the probable occurrence of destructive numbers of the pests. Warned by their abundance, the Station Rome orchard was sprayed with the nicotine-lime wash as a protective measure. The application was made on June 12 and 13. At this date breeding of the lice was abundant, but there were no injuries except an occasional curled leaf.

Test II. This experiment was a comparative test of the nicotine sulphate-soap and nicotine sulphate-lime combination sprays on young trees. The sprayed block comprised 130 trees about eight years of age, several of which had commenced to bear fruit. The mixtures were applied on June 12 and 13. The foliage injury at this date was unimportant.

Results of the experiment.— In both tests the protection from the outbreak afforded by the lime was striking. The nicotine sulphate-soap application destroyed the aphids originally present, but was of no value as a protective agent. The trees sprayed with the

soapy solution were eventually reinfested, and at the peak of the outbreak were not different from the checks in appearance. The rows in each orchard that received the nicotine-lime combination spray were quite free from lice when they were most numerous on check trees, and the coating was sufficient to act as a deterrent to the insect activities on the trees. The foliage in each instance was flattened and luxuriant, which compared favorably with the sooty curled leaves of trees in the unsprayed block. The new fruit buds on the checks in the older planting became stunted. In September they were undersized, and lacked the vitality of the plump, normal appearing buds of the lime-sprayed Romes. These characteristic differences are shown in Plate XXIV.

The benefit from the treatment to the larger bearing trees was chiefly noticeable at picking, when the check apples were sticky, blackened and streaked like the fruit in Plate XX. The apples from the lime-sprayed block were larger as a rule at the time of harvesting than the checks, and entirely free from discolorations similar to those on the untreated apples. The single spray on the young trees warded off the destructive attacks of the lice until late in July. After that period, and as the unprotected new growth unfolded, much of the benefit of the application was lost. This experience afforded an excellent illustration of the number of treatments young, luxuriantly growing trees may require if the first application is made as the lice appear on the trees in June. The more extended period of immunity of leaves and fruits on the older trees was possibly owing to their size and heavy foliage, which hindered the washing effect of the heavy rains of early summer.

CONTROL OF INSECT IN ORLEANS COUNTY ORCHARDS.

STATION EXPERIMENTS.

In answer to the request for assistance from apple growers in Orleans County, this Station instituted orchard experiments to ascertain whether the soap spray or lime wash would prove effective mixtures in such an emergency. In these tests, the nicotine sulphate was increased to one pint for each one hundred gallons of spray. This change from the standard recommendation was arbitrary, and was prompted by the belief that the additional tobacco would

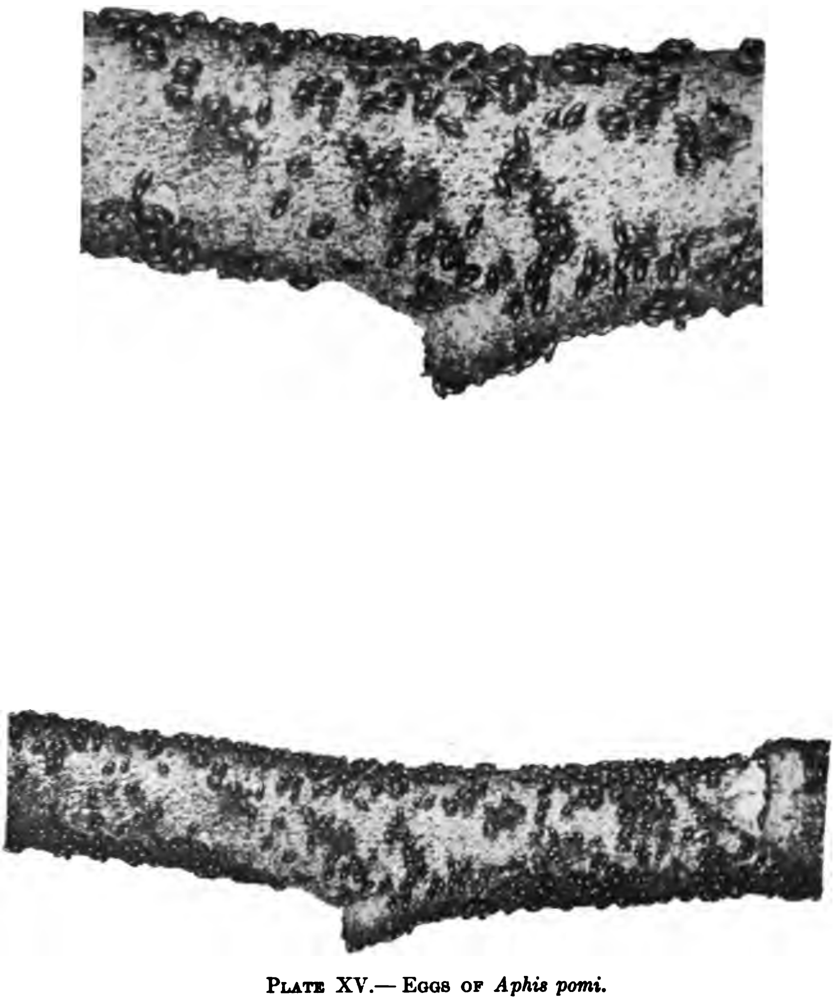


Plate XV.— Eggs of *Aphis pomi*.
Lower, enlarged; upper, much enlarged.

PLATE XVI.— CURLING OF APPLE SHOOT BY *Aphis pomi.*

PLATE XVII.— GREENING APPLES SHOWING CHARACTERISTIC STUNTING OF FRUITS BY *Aphis pomi.*

PLATE XVIII.— MISSHAPED TWENTY-OUNCE APPLES SHOWING ROUGHENING OF SURFACES.

PLATE XIX.— TYPICAL MALFORMATIONS OF BALDWIN APPLES DUE TO *Aphis pomi*.

Plate XX.— Rome Apples Showing Effect of Honey-dew Fungus on Leaves and Fruits.

PLATE XXI.— RED STIPPLING OF GREENING APPLES BY *Aphis pomi*.

PLATE XXII.—SPRAYING IN THE DONAHUE, MCCRILLIS, MACK CO. ORCHARD, HOLLEY.
Most efficient method of treating large trees.

PLATE XXIII.— LOWER, RESULT OF APHIS WORK ON FRUIT CLUSTER. UPPER NEW GROWTH STARTING ON TERMINAL AFTER SPRAYING.

Plate XXIV.— Upper, Unsprayed Rome, Showing Stunting of Fruit Bud. Lower, Bud Development on Rome Sprayed with Nicotine-Lime Wash.

appreciably aid in ridding the trees of the older lice which might be able to recover from the toxic effects of the weaker spray.

Test I.— The two sprays were applied to twenty-one trees in the orchard of the Donahue, McCrillis, Mack Company, Holley. These trees are about fifty years of age, unusually high, and have wide-spread branches. The foliage was dense, and was severely injured by the insects. When the sprays were applied on July 19 the apples were black and roughened, and the new fruit buds were backward in development. The lice were so plentiful that on some fruits the color was obscured.

To wet thoroly both surfaces of the leaves, an average of twenty gallons of the nicotine sulphate-soap spray was applied to each of fifteen trees. The nicotine sulphate-lime mixture having less surface tension, more of it per tree was required on the average, and consequently only six trees were treated with the same amount of spray as was used with the preceding lot of trees. As a result of the drenching they received, both leaves and fruits were rather thickly coated with the material.

Results.—The killing properties of the nicotine sulphate-soap spray effected immediate control of the infestation. The lime-wash was somewhat slower in action, but the next day no differences in the effectiveness of either spray were noted. On the row sprayed with nicotine sulphate and soap the fruit and leaves continued to be blackened with the "honey-dew fungus." The apples and foliage of the other plat were coated with the lime, which remained well into the autumn. On September 17 a film of lime was yet adhering to the fruits, altho it was largely washed from the leaves. These trees, however, looked much more healthy than those having the soap spray. The leaves and fruit on the latter were somewhat oily in appearance. Altho they had recovered much of their normal shape there was, on the whole, a noticeable difference in the condition of the two sprayed blocks. On both plats the apples at picking time had largely become normal in size. Some trees in the orchard served as checks, and the fruit on them was mostly undersized and misshapen. As compared with these, the sprayed rows produced a large crop of high-grade fruit which, if the treatment had been neglected, would have been as worthless as the apples on the unsprayed lot.

Test II.— On July 18 forty trees in the fifteen-year-old Greening orchard of Mr. B. G. Wilson, Carlton, were sprayed either with nicotine sulphate and soap or the nicotine sulphate-lime wash. The amounts of lime used in this test were forty pounds in each hundred gallons of spray. The trees, which were well fruited, were, on this date, being seriously damaged by the lice. Aside from the curling of the leaves, the foliage and fruits were dripping with the "honey dew" and were heavily coated with sooty fungus. It was impossible to pick a normal apple, and individual trees were so badly injured that bud development was checked, and terminal growths were stunted. The amounts of materials required to thoroly drench the trees varied somewhat. On the average, there were required 8¾ gallons of the soap spray and 10 gallons of the heavy lime material respectively for each tree. This disparity was owing to the difference in the spreading qualities of the liquids, as the trees themselves did not noticeably vary in size.

Results.— After spraying operations had been completed, it was seen that the soap solution had destroyed the pests on trees receiving that application, and on the following day no lice were alive on the lime-sprayed plat. The lime coat was not as heavy as on trees treated with the usual formula, and the deposit was more film-like in general appearance. Both leaves and apples were well coated with the spray.

On July 22 both plats were equally clean. Even in the areas of most severe infestation and in curled leaves the lice were dead. On September 16 the apples had improved greatly in size, and both plats had few fruits which showed injuries from *pomi*. The lime had washed from the apples and leaves, and they were entirely free from the early discolorations. On the soap-sprayed row the dead lice yet adhered to the fruits, which were black, sticky and streaked as when the infestation was at its height.

COOPERATIVE EXPERIMENTS IN COMMERCIAL ORCHARDS.

Example I. Orchard of the Donahue, McCrillis, Mack Company, Holley.— This is the planting in which the Station experiment was located, and it comprises about 150 trees of the varieties Baldwin and Greening. The orchard was originally closely planted, and after fifty years the trees had attained considerable height, and had wide-spread branches. As the tree growth became entangled,

the alternate trees in each row were shorn of their branches, but the trunks were allowed to remain. In the course of a few years these were covered with new growth. During the past summer these trunks had become bushy trees of considerable size, and the succulent branches were so abundantly infested with *pomi* as to cause practically all the leaves to curl and drip with " honey dew." It was evident that these " stubs " were the chief centers of infestation in this orchard, and, at our suggestion, they were removed. Spraying was accomplished on July 18 and 19, using nicotine sulphate and soap in the proportions of one pint of the nicotine solution and four pounds of soap to one hundred gallons of water. The trees were drenched with the spray, and, on the average, each received twenty gallons of the solution. Owing to the extreme height of the trees, it was necessary to use a high pressure equipment to force the liquid to the tips of the highest branches. The interesting feature in these operations was the use of two spray guns instead of one as is ordinarily practised. (Plate XXII.) This outfit made it possible to spray beneath the limbs and on the outside branches simultaneously, thus making a thoro application without causing undue delay.

The results of the treatment in this planting were immediately noticeable, and on July 20 no insects were alive in the sprayed section. By July 27 the trees had begun to recover from injuries, and new leaves were unfolding on terminals that had been previously injured by the aphids. While dead insects and " soot " adhered to fruits and foliage, no new infestations were found. The apples for the most part overcame the lesser injuries so that, at an examination on September 16, such fruits were well developed and had lost the initial deformities. As a rule the fruits were of excellent size, and the crop of apples was one of the best in the section. What might have occurred had no spraying taken place was well illustrated by several trees which were quite difficult to treat. These at harvest bore apples of only inferior grade and size, many of which were typical *pomi* fruits.

Example II. Orchard of Dayton True, Holley.— In this planting, 183 Duchess and Baldwin apples were being severely attacked by *pomi* early in July, and at that time it was estimated that 25 per ct. of the fruits were injured. Individual trees between forty and fifty years old had been pruned so that the branches did not overlap to any extent. Other trees of the same age and variety had received

very little attention in this respect, and the woody growths were more or less intertwined. Water-sprouts were abundant under these conditions, and the foliage was dense. In such instances, the attacks of *pomi* were causing serious injuries to apples on July 7, and the leaves were sticky and discolored. The heavily pruned trees with more open foliage were less injured by the aphis, and it appeared that, in this planting at least, air drainage was a considerable factor in the extent of the infestation by the lice. Water-sprouts under these conditions had comparatively few centers of infestation. Spraying was begun on July 7 with nicotine sulphate and soap in the proportion of one pint of the nicotine and four pounds of soap to one hundred gallons of water. The plan of treatment adopted was to spray only the trees or those portions of a tree where the fruits were being attacked. The aphidicide was applied each day as was convenient. On July 22 only dead lice were found on the sprayed trees, and the work had been so thoro that even lice in curled leaves were destroyed. The fruits, especially those of the earlier maturing kinds, exhibited considerable roughening on the surfaces, and many were abnormal in shape, and pimpled. On September 16 the apples had largely recovered from injuries inflicted by the aphids, altho the surfaces of the apples in a number of instances were pitted. Visually, such fruits did not exhibit abnormal characteristics, and the blemishes were detected only by the sense of touch. The fruit at picking time failed to show many of the earlier deformities, and the ultimate losses were comparatively small.

Example III. Orchard of D. A. Salisbury.— There were 375 Baldwin trees in this orchard, the majority of which were about forty years old. As is common in plantings of this age, the trees had attained an unusual height, and had wide-spread branches. The foliage was luxuriant, and on it the lice were breeding in remarkable numbers. So abundant were the aphids that the leaves, twigs and fruits literally dripped with "honey dew," and had become coated with the "fungus." On July 16 and 17 about 250 trees were sprayed with the nicotine sulphate-soap solution, using one pint of the tobacco extract and four pounds of soap in one hundred gallons of water. The trees were thoroly drenched, and within a few hours after the application an examination showed only a very small number of living aphids. On July 22, in addition to an improved condition of the orchard, the fruits themselves appeared

to have recovered to some extent, and their uneven surfaces were becoming smooth. By harvest the apples generally had become of good size, and an earlier crop estimate of 10 per ct. shrinkage, owing to lice injury, appeared much too large. Fruits which were pimply had become smoother, but they were not entirely free from blemishes. Apples which were severely injured did not entirely recover from the effects of the insects' attacks, and at the time of picking were thrown out as culls.

Example IV. Orchard of Mrs. George H. Rolfe, Lyndonville.— In this planting some 70 Baldwin apples were badly infested with *pomi.* The trees, which were about forty-five years old, had been allowed to grow until at the present time the height and interlacing of branches has made it difficult to spray them thoroly. The work of the aphids on leaves was not as severe as in some of the other plantings. The fruits, however, were being attacked, and restricted areas of the foliage bore a sickly appearance. The lice seemed to be increasing in numbers on July 19, and, as a precautionary measure, the orchard was sprayed, using the nicotine sulphate-lime mixture as the aphidicide. Owing to the conditions of the infestation, special attention was given to those parts of the trees which seemed to be the most severely infested. The amount of wash necessary to cover the trees in this manner was 1600 gallons, which averaged 22.8 gallons per tree. When the orchard was examined on July 22 it was estimated that from 85 per ct. to 90 per ct. of the aphids were dead. At harvest the benefit of the whitewash was apparent in the clean appearance and high color of the fruits. Apples from untreated trees were at that time distinctively sooty, sticky and discolored, and in general were below the normal size for the variety.

Example V. Orchard of E. M. Mower, Carlton.— This orchard comprises about 500 Baldwin trees of good size with luxuriant foliage. Owing to the great abundance of the lice, serious injuries were showing on the apples when they were treated early in July. Some doubt existed on the part of the orchardist as to the practicability of sprays on trees of that size, and only one-half of the planting was sprayed. The balance of the orchard received no treatment. The sprayed section was treated with the nicotine sulphate-lime combination spray, which was used in liberal amounts and very carefully applied. When this orchard was examined on

September 16 the difference in the two parts of the orchard was particularly striking. Apples and foliage on the treated section were clean, and showed but slight traces of aphid injury. On the unsprayed trees the apples for the most part were pimpled and distorted, and the remainder of the crop was undersized. The crop on this plat was practically worthless as the apples did not attain normal size, and the flesh, even of those not noticeably affected, was quite corky in texture. The foliage was black and discolored, and the buds on fruit clusters were undeveloped. The loss occasioned by the failure to make a complete spraying was heavy from a financial viewpoint. On the other hand, an orchardist is seldom given so apt an illustration of the benefits which may be derived from properly timed applications of insecticides where the work is entirely within his control. The results from his own test led Mr. Mower to state emphatically that he was convinced that the entire planting should have been sprayed, and that hereafter he will not hesitate to treat the trees, using the thickened wash as the carrier for the nicotine solution.

OPINIONS OF ORCHARDISTS ON THE SPECIAL SPRAYING.

The application of experimental results to orchard needs is most satisfactorily attested by growers in adopting these newer ideas into spraying practice. The extensive spraying operations by many apple growers in Orleans County against the green apple aphis provided an excellent opportunity to ascertain if the work accomplished in the experimental plats could be repeated under common orchard conditions, or was considered practicable by individual growers. To collect these facts, the Orleans County Farm Bureau issued a questionnaire to be filled out in connection with the regular spraying service report. Nineteen fruit growers reported benefits from spraying, three men made no statement, and one grower reported that the application was detrimental. It is interesting to note in this connection that of those reporting success, eight orchardists were so confident that benefit had been derived that they offered an estimate of the increased value of the apples as a result of the treatment. The reports showed that the operations were conducted without financial losses, and all but one report showed a wide margin of profit.

COMPARATIVE COSTS OF SPRAYING.

The adoption of a regular spraying program depends in large measure upon the outlay for materials and labor. Some growers, thru the exercise of an unwise reliance on the element of chance, sometimes appear to secure large returns for a minimum outlay on spraying. Men who have obtained the greatest success from a financial viewpoint rarely adopt such a precarious program, but aim rather to grow clean apples regardless of the initial expense if it is within reason. To such men, an additional spraying for the green apple aphis was a matter of insurance in which the premium was expected to be amply repaid.

As previously stated, two types of aphidicides were used in the experimental efforts of the season. The nicotine sulphate-lime combination, besides being a killing agent, acted also as a repellent. The nicotine sulphate-soap solution was relied upon for immediate results, especially on trees of great size. The expense connected with either spray depended entirely upon the sizes of the trees and the proportion of smaller ones in plantings of older apples. Obviously some large trees bore a greater initial expenditure than younger or lower-headed trees. The outlay for generous applications to the large trees has been deemed by different growers as excessive. The use of quantities of a contact spray sufficient to secure positive results necessarily does increase the cost, but not usually in proportion to the benefits secured from the treatment. To illustrate the spraying outlay under average orchard management, the expenses in the sixteen orchards and smaller plats in Table II will serve to show the distribution of the charges against the special treatment in representative plantings.

It will be seen that a wide variation exists in the expense of spraying. The individual equation largely functions in these seeming discrepancies, and is responsible in a measure for failures to check insect outbreaks. Despite the differences in amounts of spray applied per tree, these orchardists claim to have controlled the aphids; but it is also evident that on trees of similar size the application of large amounts of spray must have produced a wider margin of safety than where minimum dosages were applied. In the Station experiment (No. 10) the excessive cost of the lime was due to

the extreme size of the trees and the severe infestation which required an extra careful treatment.

TABLE II.— RELATIVE COSTS OF SPRAYING MIXTURES.

Number of orchard.	Number of trees.	Age of trees.	Materials used.	Cost of spray and labor.†	Cost per tree.
1.......	325	6–50	Nicotine sulphate-lime.....	$23.00	$0.04–$0.13
2.......	104	10	" " "	16.87	.16
3.......	75	35–50	" " "	12.00	.16
4.......	110	6– 8	" " "	2.25	.02
5.......	538	8–60	" " "	64.15	.12
6.......	80	80	" " "	15.00	.18
7.......	275	30–60	" " "	75.00	.27
8.......	240	35	" " "	80.28	.33
9.......	70	45	" " "	48.02	.69
10*......	5	50	" " "	6.79	1.36
11*......	20	13	" " "	4.86	.24
12*......	20	13	Nicotine sulphate-soap.....	3.80	.19
13.......	375	40	" " "	50.00	.13
14.......	183	40	" " "	61.80	.34
15.......	150	50	" " "	75.00	.50
16*......	15	50	" " "	5.55	.37

* Station experiments. † Including team hire.

PRACTICABILITY OF SPRAYING.

To supplement the figures on cost of operations, a personal letter was sent from this Station requesting opinions of fruit growers on commercial factors entering into the problem of aphis control which could be published with this bulletin. From eight replies the following excerpts have been taken:

Letter No. 1.— Mr. Dayton True, Holley, writes: "During the week of July 7, after I had applied the fourth regular spray, the aphis began to appear in such quantities as to seem a serious menace. I sprayed with the usual formula of nicotine sulphate and whale-oil soap, adding arsenate of lead for the benefit of such stray codling moths as might appear. I gave the trees a thoro drenching, using two leads or nozzles, one playing underneath the trees, the other from the tower above, drenching so far as possible contra to each other. From what I could see we killed about 90 per ct. of the aphis by this method. I noticed the trees that are openly trimmed were much less affected than the thick, bushy trees. I

noticed that the ground suckers and main crotch suckers are the starting point for these aphids in every tree, and the deduction to be drawn is obvious."

Letter No. 2.— Mr. Dan A. Salisbury, Holley, replied as follows: "About June 1 the aphis was first noticed in our orchard, and appeared only in some parts, but not covering the orchard as a whole. Not knowing what to do, I used as strong a solution of lime-sulphur as I dared. This had no noticeable effect in checking the spread, and finally, upon close inspection, I found the young fruit literally covered with the green aphids. Upon the advice of Mr. Steele, our county farm bureau agent, I used a solution of 2 pounds vitriol, 1 pint nicotine sulphate and 40 pounds of lime to 50 gallons of water. I think that this spray helped to check the aphis, but about two weeks later I used a solution of whale-oil soap and nicotine sulphate, 10 to 15 gallons to each tree, and the results of this spray were wonderful. This spray was applied on July 4. I harvested a crop of 1608 barrels of Baldwins, three-fourths of which would classify as No. 1. I am thoroly convinced that, had I not checked the aphids, my crop would have been practically ruined, and I attributed the saving of the apples to the close cooperation of the Experiment Station and the Farm Bureau."

Letter No. 3.— Mr. Thomas Mack, Holley, replies: "The green aphids were getting the best of my apples when I started to spray about the middle of July with lime and nicotine sulphate. The trees in my orchards are so tall that it took too much of this material to cover them, and after spraying several I adopted the tobacco solution with soap, as was recommended. Either spray will kill the lice. I think the lime and tobacco would work better on smaller trees. The spraying destroyed about 75 to 80 per cent of the lice. In fact, they were so well cleaned out that the apples and trees recovered, and very few aphis apples were thrown out of the barrels. The crop was excellent, but the few apples sprayed with lime were somewhat coated at picking time. I would not want to use it on a whole crop. Another year I would spray earlier, using the soap and tobacco, which covers easier and kills quicker on trees the size of mine. My success was due mostly to the use of the large sprayer. In our orchards an ordinary rig does not give enough pressure to spray the tops of the trees. With lots of

pressure and drenching of the trees there can be no question as to the control of the lice. In my experience spraying certainly paid."

Letter No. 4.—Mr. B. G. Wilson, Waterport, sent the following interesting letter: "We sprayed four varieties of apples: Greenings, Baldwins, Wealthy and Duchess of Oldenburg. The trees are 13 years old. Regular sprayings were made, first, when dormant, with lime-sulphur 1 to 8; second, in the pink, using lime-sulphur with nicotine sulphate; third, the calyx spray; and fourth, two weeks later. After that the green aphids came. We sprayed, using the Station formula for nicotine sulphate and excess of lime. Where thoroly done, this is very effective. Nicotine sulphate and soap is good. I haven't the cost of spraying for aphis, but it is very small in comparison with the results, as our apples were entirely free from lice injury. I certainly would spray again if we should have the green aphids, using nicotine sulphate and lime as soon as I thought they were coming in any quantity. The story was circulated that my crop was spoiled, but I never had such a fine crop of clean apples as I picked this year."

Letter No. 5.—Mr. Ora Lee, Albion, sent the following description of his spraying operations: "Early in July the lice appeared in considerable numbers on a 5-acre, 5-year-old orchard of Greening and Twenty Ounce apples, and on the insides of old bearing Greening trees; also a few on Baldwin and others in a 4-acre orchard. They gradually increased until on July 16 I deemed it advisable to spray. At that time the young trees and new wood on insides of old Greenings were quite seriously infested. We applied three tanks (300 gallons each) of spray as follows: 300 gallons water, 3 pints nicotine sulphate, 6 pounds copper sulphate, 150 pounds lime, thoroly drenching the young trees and giving particular attention in the old orchard to the insides, especially the water-sprouts. The following day, after a very careful inspection, I estimated that over 95 per ct. of the lice were killed in both orchards. They did not reappear in sufficient numbers to cause any worry. We got them before they had caused apparently serious injury except to fresh growth of water-sprouts in the shady interior of old Greenings which, in some instances, they trimmed completely. After the spraying they did not get to the fruit or fruiting parts of old trees in serious numbers, and I could not at any time see that they injured the apples to any appreciable extent.

In fact I credit this and the pink spray which I applied this season, for the first time, for the best quality apples I have ever had. Two or three years ago I had a quite serious infestation in the young orchard, and at that time on the smaller trees got at least 99 per ct. of them with the old lime-sulphur-nicotine sulphate mixture, so that, from my own observations, I am inclined to the idea that success in catching green aphis depends very largely on the ability to hit the lice with nicotine extract regardless of what it is mixed with."

Letter No. 6.—Mr. Robert Burke, Kendall, writes: " We sprayed our 9-year-old apple trees with the nicotine sulphate-lime mixture for green aphis on July 17 and 18. We drenched the trees and killed the aphids with apparently good results to both fruit and foliage. The apples were very fine when picked, and the leaves on the trees were healthy. The material used was 60 pounds hydrated lime, 1 pint nicotine sulphate, 2 pounds blue vitriol, 100 gallons water. The wash was applied with a spray gun."

Letter No. 7.—Mr. William Thiel, Lyndonville, writes: "I can safely say that 60 pounds lime and 1 pint nicotine sulphate to 100 gallons water will control the green aphis. I used this in one of my orchards with good success, just spraying from one way from the ground. I could not get any more lime so I used the nicotine sulphate with the lead and lime-sulphur spray, working from both sides with but little success. I will recommend lime and nicotine sulphate above everything else which I have heard of as yet. It also took the place of the usual July scab spray. From my experience I consider this spray beneficial to the orchard."

Letter No. 8.—Mr. Clarence F. Powley, Lyndonville, replied: " In regard to the green lice last summer, I used nicotine sulphate, lime-sulphur and 32 pounds of lime to 100 gallons of water. The next day I examined some of the trees that were sprayed the day before and I am satisfied that 75 per ct. of the lice were killed. They would have ruined my crop of Greenings and Kings if allowed to continue feeding. As it was, there were 540 barrels of Greenings and 100 barrels of Kings. I can recommend the nicotine sulphate and lime as an aphis spray."

DISCUSSION OF RESULTS AND SUGGESTIONS FOR FUTURE SPRAYING OPERATIONS.

It was apparent that of the two sprays under consideration the nicotine sulphate-soap solution was the more quickly active. After a few hours from the time of application, no difference in the effectiveness of the two mixtures was noticeable on well-sprayed trees. On account of its greater spreading properties and rapidity as a killing agent, many growers prefer the nicotine sulphate-soap spray, especially on trees of great height and with wide-spread branches.

Small bearing trees sprayed with the soap solution were gummy and black at harvest. This was not as noticeable on trees of great size, and it appeared that the wealth of succulent stems and leaves was responsible for a situation on the younger stocks which was almost beyond comprehension. In the Station test on 13-year-old Greenings the fruits on those treated with nicotine sulphate and soap were no cleaner in September than unsprayed trees in the same orchard.

On trees of moderate size and of an age less than 25 years the nicotine sulphate-lime spray was not only efficient, but the coating served as a repellant, and prevented the harboring of lice, especially on the more tender foliage. Small trees protected in this manner did not produce the bushy terminals common to aphid activities. Older fruiting apples also exhibited a similar freedom from the stunting by the insects, and in addition the fruits were clean and free from the sooty, sticky accumulations that usually accompany attacks by lice. In certain instances mechanical difficulties were responsible for a reduction of the amount of lime in the formula. When thoroly applied it was found possible to decrease the quantity of hydrated lime to 40 pounds in each 100 gallons of water and secure good aphis control on young trees.

A number of growers claimed that the nicotine sulphate-lime wash did not cover the foliage of tall trees as well as nicotine sulphate and soap and not only was more difficult to apply but the actual spraying of a tree took more time. Cost of material entered also into the considerations. From our experience in actual orchard operations on very large trees, these criticisms appear to have been well founded altho the cleansing effect of the lime should have received more attention.

Owing to the greater ease of application and its covering properties, orchardists judge that the nicotine sulphate-soap solution will be most generally used on trees of extreme size. The range of usefulness of the nicotine sulphate-lime wash, due largely to its added cost and lesser surface tension, is apparently restricted to trees of moderate age. Its field is more that of a deterrent and cleansing agent than of a strictly contact remedy. The efficiency of either aphidicide is not questioned.

The value of the delayed dormant application is indicated only, and, while plantings having this treatment were apparently better able to withstand the onslaught, this early spray cannot be depended upon owing to the migratory habit of the aphids in late spring.

Orchard operations showed conclusively that important injuries from the green apple aphis may be practically obviated by proper spraying. These tests also served to crystallize the opinions of apple growers as to the comparative merits of special sprays for the control of this pest as well as to demonstrate more clearly than heretofore the conditions under which spraying should be done to obtain efficient results.

REDUCTION OF SPRAYING LOSSES.

Fruit growers have been taught that a mist-like spray is proper for making the usual applications in the spraying schedule. While such treatments with poison sprays are, when carefully made, quite efficient, it is often necessary to use coarser drenching sprays against certain insects, as aphids, pear psylla, etc. Insufficient quantities of spray are often a waste of materials, altho in some instances the failures to control an insect are due as much to lack of care in making the applications as to inadequate amounts of material. Spraying with the nicotine sulphate-lime wash provided a number of interesting examples of such oversight. In these instances the trees, instead of having a whitewashed appearance, were streaked, and invariably such efforts to control lice were ineffective.

Difficulties in covering trees of great size provided an excuse for fruitless attempts in *pomi* control in old orchards. If the season's experiences are reliable criteria, there appears to be little justification for such criticisms. It has seemed that, wherever the men doing

the work were correctly informed as to the purposes of the spraying and the activities of the insects, greater interest in the work was aroused, which invariably led to more effective results. Machines with insufficient power to deliver a large volume of liquid or unsatisfactory types of nozzles were probably larger factors in spraying than was generally suspected. Orchardists who gave little attention to these various details often met with failures to control the green aphis. An indispensable factor in efforts to combat aphids, however, is efficient machinery, which, in a measure, will overcome many unpleasant features connected with ordinary spraying operations as well as render more effective service.

TIMING SPRAYING OPERATIONS.

The proper timing of spraying operations against the green aphis is puzzling to many apple growers. The outbreak of 1918 would in all probability have been checked in its incipiency if control measures had been adopted at the outset. It is difficult to judge in early June the extent to which the later breeding of *pomi* may become a serious factor in orchard economy. Probably in most seasons spraying of older bearing apples for this pest is unnecessary, altho in young plantings some treatment might be profitable.

The flight of winged migrants during the last week in May or the early days of June is a good indication of the approaching spraying period. To prevent injuries to the tender stems of young trees, an application of the deterrent wash at this time will be useful. The lime mixture should be repeated as the newer terminal growth appears, and unprotected leaves become reinhabited by the lice. In older bearing orchards the infestation of terminals and leaf clusters serves as an indication of unusual breeding of the insects, and applications of the repellent spray under such conditions may prove helpful. If, however, the green aphids begin to attack the fruit clusters, the work should not be delayed, altho from observations during 1918 it appears advisable to wait until the lice begin to feed on the stems and young fruits before starting spraying operations. In any circumstance the grower should not wait until the apples are gummy or blackened before securing reliable instructions as to the advisability of making the application.

SPRAYING MIXTURES AND FORMULAS.

Formula 1. Nicotine Sulphate-Soap Solution.

Nicotine sulphate (Black Leaf 40)	¾ pint*
Soap	4 pounds
Water	100 gallons

Formula 2. Nicotine Sulphate-Lime Wash.

Stone lime	60 pounds†
Copper sulphate	2–4 pounds
Nicotine sulphate (Black Leaf 40)	¾ pint*
Water	100 gallons

Slake the lime slowly to make a good milk of lime free from coarser particles. Causticity may be lessened by preparing the lime a day before using. The dissolved copper sulphate should then be passed into the diluted lime wash, after which the diluted nicotine should be added. If the attacks of lice are severe, increase the amount of nicotine sulphate to one pint.

DIRECTIONS FOR SPRAYING.

Rather coarse sprays in copious quantities are essential in order to obtain efficient control of the green aphis. With the lime wash, especial care must be taken to coat both surfaces of the leaves as well as the young apples, or else the grower will not obtain the greatest benefit from this treatment. Thoro applications of either spray are necessary as the insects must be hit by the liquid, or killing will not result.

CONCLUSIONS.

The green apple aphis lives on apple trees thruout the year. On account of a late spring migration of winged forms and the later breeding of the insect, the pest is difficult to control by a single spraying in the season. If control measures are unduly delayed the insect activities may result in severe injuries, as curling of leaves or deforming of fruits.

Curled foliage and the stems of fruits as well as the clusters of apples afford hiding places for the insects, which are difficult to reach by the spraying mixture unless it is applied generously and with considerable force. Applications of coarse sprays in liberal quantities are necessary to thoroly wet the leaves and the insects.

* During periods of severe attacks increase the amount of nicotine-sulphate to one pint.

† Hydrated lime may be substituted.

Such treatments often reach aphids that escape mist sprays, and by thoro and timely spraying the summer broods of the lice can be controlled even on trees of considerable size.

The delayed dormant spray, by protecting the trees from early infestations of the lice, diminishes the opportunities for serious reinfestations from late spring migrations of the insect.

The nicotine sulphate-soap spray is a very satisfactory aphidicide on account of rapidity in killing, ease of application, and its spreading and adhesive properties.

Nicotine sulphate and lime is especially advantageous on trees of medium size with large amounts of succulent growth because of its deterrent influence on the insects in addition to its immediate killing properties.

In planning spraying operations against the green apple aphis, chief dependence should be placed on the nicotine sulphate-soap spray for trees of unusual height. With plantings of younger trees or those newly set, especially where succulent stems are likely to be seriously injured, an application of nicotine sulphate and lime will prove an efficient and satisfactory treatment.

THE ROSY APHIS IN RELATION TO ABNORMAL APPLE STRUCTURES.*

P. J. PARROTT, H. E. HODGKISS AND F. Z. HARTZELL.

SUMMARY.

Apples attacked by the rosy aphis usually display suppression of the transverse and axial diameters. Inhibition of growth occurs to a greater extent with the transverse diameter. The injury varies in extent, even with fruits of the same cluster, and the amount of damage is largely determined by the earliness and intensity of attacks and the duration of period of infestation.

Besides being reduced in size, affected apples are frequently poorly colored and are often not symmetrical in shape, one side being undeveloped, which causes the fruits to present a lopsided appearance.

With apples showing extreme contortion one or more carpels on the side of greatest suppression in development frequently lack seeds or contain seeds that are inferior in size and weight to those of complementary cells.

Seeds vary in number in normal and aphis-injured apples. Fruits not subjected to attacks by the insect surpass, on an average, aphis apples in number of seeds per apple. Injured apples show greater variability in seed content than do normal fruits. Apples under 30 mm. in transverse diameter are often sterile or have only a small number of seeds.

Attacks by the rosy aphis tend to reduce the extent of dropping of fruits which possess empty carpels or have only few seeds. The existence of sterile or few-seeded apples on trees at the period of harvesting has the effect of lowering the mean number of seeds per apple and of increasing the variability of seed content.

During 1916 apples had a greater range and a higher mean number of seeds per apple than fruits of the following year. This condition was noted with both normal and aphis apples, but with normal fruits the difference in variability was negligible.

The average weight of seeds per apple was greater with normal fruits, and in comparison with aphis apples during the past two years the difference has been marked. The variability in weight of seeds was also much greater with injured fruits. In normal fruits the average weight of seeds per apple and the range in weight were greater in 1916 than in 1917.

* Reprint of Technical Bulletin No. 66, January, 1919.

As judged by size, plumpness and appearance of integument, both classes of fruits contained imperfect seeds. These were more abundant in aphis-injured fruits.

As selected, normal apples weighed more than aphis apples. During 1916 they averaged 2.6 times the weight of aphis-injured fruits and during 1917 they were 3.2 times heavier. The coefficients of variability both seasons were considerably greater with the injured than with the normal fruits. Notwithstanding differences in average weight of seeds, normal apples weighed approximately the same for both seasons.

Injuries by the aphis did not affect all structures of the apple to the same degree. The greatest per cent of decrease occurred in the weight of the individual fruits, with a smaller decrease in the weight of the seeds and the least decrease in the number of seeds.

A comparison of variability in the several structures of normal apples showed that the variation in the number of seeds per apple is the most marked, followed by weight of seeds, while weight of fruits is the least variable. In aphis apples, however, the variability of each character is greatly increased over that found in normal apples, weight of seeds being highest, number of seeds intermediate, and weight of fruits least variable.

The coefficients of correlation between weight of seeds and number of seeds per apple in aphis-injured and normal fruits both seasons were positive and high, but the regression coefficients of the two series each season did not differ to any great extent.

There appears to be a closer relationship between fruit weight and seed weight in small apples than in large ones, but in none of the series is the relation between fruit weight and seed weight very marked.

Severely malformed fruits sustained no reduction in the number of primary fibro-vascular structures. The strands on the side of greatest distortion generally showed suppressions in development and displacements with respect to distance from the vertical axis of the apple and the amount of space between the different elements. The ultimate branchlets did not display the dense plume-like appearance which is characteristic of normal, ripened fruit.

Irregular developments in form and structural arrangements of fruits occur independent of the work of the rosy aphis. Attacks of such apples by the insect tend to aggravate the distortions.

INTRODUCTION.

In preceding bulletins of this Station the effects of attacks by the rosy aphis (*Aphis sorbi* Kalt.) on the development and conformation of apples have been noted. In addition to inhibition of growth and asymmetrical development of fruits injured by the insect there are irregularities and perversions of form of various structures that are

especially noticeable with certain internal organs, as the seeds and the fibro-vascular system. As there is an essential relationship between the development of the seeds and the growth of its flesh it is believed that a wider knowledge of the facts as to the influence of the rosy aphis on the normal organization of apples is desired, especially by those interested in the anatomy of pome fruits and in experimental genetics in the horticultural field.

In the preparation of this bulletin the statistical treatment of the data has largely been the work of Mr. F. Z. Hartzell. The manuscript has been read by Dr. J. Arthur Harris of the Station for Experimental Evolution, Cold Spring Harbor, L. I., for which courtesy we are under obligation.

ACTIVITIES OF ROSY APHIS ON APPLE TREES.

SOME HABITS OF THE INSECT.

Hatching of the eggs of the aphis occurs during the period of the swelling and breaking of the apple buds. In most seasons the majority, if not all, of the nymphs will have emerged from the eggs by the time that the tips of the leaves have projected as much as one-fourth to half an inch from the ends of the more advanced buds. The insects creep down among the young tender leaves as they unfold. They display a tendency to remain with the leaves that they first attack and not to advance to the tips of the more succulent shoots of the leaf clusters as seems to be characteristic of associated species of aphids. A few days before or about the time that the pink color shows in the unopened blossoms the aphids of the first generation commence to mature and give birth to offspring, which marks the beginning of the appearance of the second generation of the insects. At the time of the dropping of the petals the more advanced individuals of the second generation reach maturity and enter on a period of reproductive activity. Owing to the long breeding season and rapid development of the offspring there is an intermingling of the second and third generations. Altho the first external evidences of injury are indicated by the curling of leaves before blossoming, the destructive capacity of the rosy aphis is generally not fully indicated until the maturing of the second generation and the appearance of the third generation, when there is usually a noticeable invasion of leaf and fruit clusters in the vicinity of the primary centers of infestation. While alate individuals may be noticed in the second generation, the maturing of the third generation is characterized by the appearance of winged forms, and about the middle of June these begin to abandon the apple trees, the migrants seeking plantains as the summer hosts. After this period the numbers of the insects on apples begin to diminish. By the latter part of July the apples are for the most

part, if not entirely, free of the rosy aphis. The dates of the first appearances of the nymphs of the different generations and periods of infestation of apples during the past four years are given in Table I.

TABLE I.— EXTENT OF DURATION OF ATTACKS ON APPLE TREES DURING 1915–1918.

	1915.	1916.	1917.	1918.
Date of hatching of first generation..	Apr. 16	Apr. 22	Apr. 20	Apr. 17
Date of first appearance of nymphs of second generation............	May 3	May 10	May 26	May 11
Date of first appearance of nymphs of third generation...............	May 29	May 29	June 11	May 17
Date of final disappearance of aphis from apple trees.................	June 22	July 30	Aug. 4	July 31
Length of period of infestation of apple trees.....................	68 days	100 days	107 days	106 days

INFLUENCE OF APHIS ON FOLIAGE AND FRUIT.

Of the various species of aphids that exist on the apple tree, the rosy aphis is conspicuous for its partiality for the foliage of blossom and fruit clusters and by reason of this preference the presence of large numbers of the insects on the host is generally attended with damage to both foliage and fruit. (Plate XXVII.)

Injuries to foliage.— The first evidences in the season of the destructive work of the rosy aphis is indicated by a severe and characteristic curling of the leaves, which may be detected just before blossoms open. An individual aphis is capable of curling one leaf tightly, but several of the insects may exist in concealed positions on a crinkly, deformed leaf. The nymphs of the second generation which appear at this time show little inclination to migrate. As a rule they remain on the curled leaf occupied by the stem mother until it becomes overcrowded with the developing aphids; when the migrating nymphs invade adjoining leaves. With the rapid multiplication of the insects the leaves that constitute the primary centers of infestation gradually become weakened by the continued feeding, frequently turning yellow or becoming otherwise discolored; and later these may drop to the ground. The insects excrete a sweetish liquid, known as "honey-dew," which favors the development of sooty fungus (*Fumago vagans* Fries). When abundant this is believed to exert to a certain extent a devitalizing influence by checking the respiratory activities of the affected portions of the plant, but generally it is perhaps not especially detrimental to fruit and leaf

clusters except as it mars temporarily the appearance of the fruits. However, the most serious consequence of a long continued attack by the aphids is that the abstraction of sap by myriads of the insects constitutes a serious drain on the vitality of the fruit spurs which often results in more or less extensive defoliation, accompanied with appreciable weakening, if not destruction, of them. The effect of a serious infestation is usually manifested by lower fruit yields, and in occasional orchards the trees do not fully regain their normal conditions of health and become productive until several years have passed.

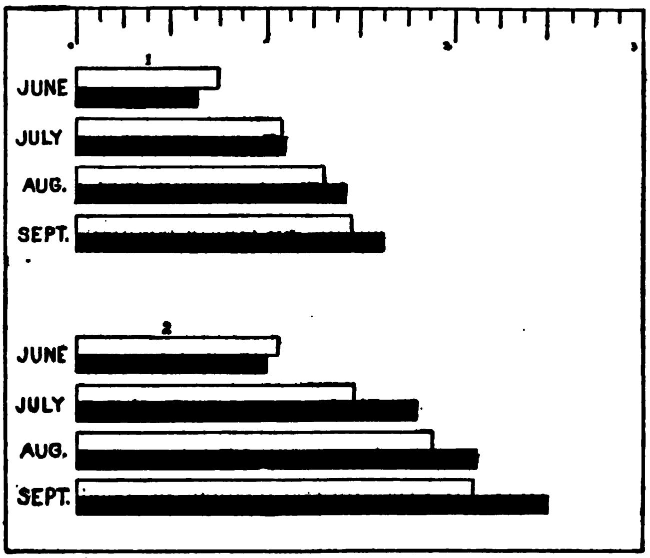


FIG. 9.— DIAGRAM SHOWING AVERAGE GROWTH OF INFESTED AND SOUND ROME APPLES.

1, Aphis series; 2, checks. *White bars*, axial diameter; *black bars*, transverse diameter.

Injuries to fruit.— The apples from clusters that have been subjected to attacks by the rosy aphis are usually checked in their development, and diminution in size is, broadly speaking, proportionate to degree of infestation and extent of duration of the attack. Therefore inhibition of growth of varying intensity may be observed among the fruits harvested from the same tree. While an aphis-injured apple will reveal reduction in both diameters, the dimensions of the transverse diameter is inhibited to a greater extent proportionately than those of the axial diameter. The average growth of apples subjected to varying injury is shown in Fig. 9, which diagram is based on measurements of affected fruits during the past four years.

Another conspicuous feature of the work of the rosy aphis is the effect of an attack on the conformation and color of the fruit. This may be clearly shown by comparison of sound and aphis-injured Rome apples, which variety has been largely used in our various studies with the insect. The fruit is normally medium to very large in size and pretty uniform in shape. In form the apples are roundish to roundish conic. The skin is thick, tough, smooth, yellow or greenish, more or less mottled with bright red which, in highly colored specimens, deepens to almost solid red on the exposed cheek, striped with bright carmine. The prevailing effect is red or red mingled with yellow. (Plate XXV.)

Injuries by the rosy aphis result in the contortion of fruits, which frequently take the form of reduction in growth on one side, resulting in a curving of the axial diameter. There is also more or less protrusion of the calyx with a star-shaped series of prominent ridges radiating from the center of the calyx end. The areas known as the cavity and basin are often abnormally shallow and undeveloped; and apples so affected frequently present a flat-bottomed or flat-topped effect. The fruits generally are poorly colored, the majority being largely green, while a small percentage have reddish tints, as shown in Plate XXVI.

EFFECTS OF INSECT ON VARIOUS APPLE STRUCTURES.

OCCURRENCE OF SEEDS IN SMALL APHIS APPLES.

In common with other varieties, a cross section of the Rome apple usually reveals five carpels radiating from the center. Each carpel contains a cell which carries the seeds. With perfect fertilization ten is considered the full complement of seeds for a normal apple, but there is a wide range of variation in these elements among sound and affected fruits. One of the effects of an attack by the rosy aphis is the presence of apples on the trees at the time of harvesting which are diminutive in size and largely free, if not devoid, of seeds. (Plates XXVIII, XXIX, and XXX.) This condition of sterility is most common with fruits of a transverse diameter of thirty millimeters or less, as will be seen in Table II, which contains the data of one hundred specimens of apples within the specified limits of size, selected at random.

TABLE II.— OCCURRENCE OF SEEDS IN SMALL APHIS APPLES.

(Counts based on 100 specimens of affected fruits; measurements in millimeters.)

Transverse diameter	6	7	8	10	12	13	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Number of apples	1	1	1	1	1	1	2	3	4	3	3	4	1	8	7	5	4	10	13	10	8	9
Number of perfect seeds	0	0	0	0	0	0	0	0	0	0	0	4	0	2	3	1	0	3	13	7	7	7

DISTRIBUTION OF SEEDS AMONG SMALL APHIS APPLES OF THE SAME CLUSTER.

In addition to differences in size, small aphis apples of the same cluster show marked dissimilarities both as to numbers and conditions of the seeds. The range of variation of five typical clusters is shown in Table III, which also gives the seed content of five normal clusters.

TABLE III.— SEED CONTENTS OF APPLES OF THE SAME FRUIT CLUSTER.

Number of Cluster.	Transverse diameter.	Number and conditions of seeds.
	APHIS APPLES.	
	mm.	
1	8	8, all shriveled.
	18	8, all shriveled.
	21	6, all shriveled.
	24	9, all shriveled.
2	13	9, all shriveled.
	31	2 healthy, 4 rudimentary.
	32	5 healthy, 3 shriveled.
	36	1 healthy, 3 shriveled.
3	16	9, all shriveled.
	24	7 healthy, 1 shriveled.
	39	3 healthy, 4 shriveled.
4	18	8, all shriveled.
	27	3 healthy, 3 shriveled.
	31	2 healthy, 1 shriveled.
5	16	4 healthy, 3 shriveled.
	22	8 shriveled.
	36	9 shriveled.
	NORMAL APPLES.	
1	52	5 healthy.
	54	7 healthy, 1 shriveled.
2	55	5 healthy.
	59	7 healthy.
	61	3 healthy, 1 shriveled.
3	56	5 healthy, 1 shriveled.
	57	3 healthy, 2 shriveled.
4	53	8 healthy.
	54	8 healthy.
5	56	8 healthy.
	58	8 healthy.

VARIATION IN SEED AND FRUIT OF NORMAL AND APHIS APPLES.

In this study there are considered the number of seeds per apple, the weight of seeds per apple and the weights of the individual fruits. As previously pointed out, the smallest aphis apples or those with a transverse diameter of less than thirty millimeters usually have no seeds or the content is very small.

The data that follow are based on affected fruits having a transverse diameter above thirty millimeters. It will be noted, however, that several of the apples above this minimum had no seeds and that these have been retained in the series. With the restriction just mentioned, all aphis apples were selected at random and are believed to be fairly representative as regards size and degree of malformation. Normal apples were selected entirely at random. Owing to the amount of labor required to collect the data in each of the series involving several characters, one hundred specimens of apples were used; but in determining the number of seeds per apple it was desired to investigate the nature of the frequency distributions in the 1917 series, and here the data were obtained from three hundred fruits.

First to be determined were the mean, standard deviation and range for each structure or character of which measurements or counts were taken and from these were calculated the coefficients of variability and the probable errors of the constants. Having these measures accurately computed, comparisons were made between the several characters and structures of normal and aphis-injured fruits.

NUMBER OF SEEDS PER APPLE IN NORMAL AND APHIS-INJURED FRUITS.

The number of seeds per apple in the several series of normal and aphis apples is given in Table IV.

TABLE IV.— FREQUENCY DISTRIBUTIONS OF NUMBER OF SEEDS IN NORMAL AND APHIS-INFESTED ROME APPLES.

Normal Apples, 1916.	
Number of seeds per apple.	Number of apples (frequencies)
16	2
15	3
14	8
13	6
12	10
11	10
10	19
9	12
8	13
7	8
6	6
5	2
4	1
	100

Aphis-Infested Apples, 1916.	
Number of seeds per apple.	Number of apples (frequencies)
16	2
14	2
13	9
12	9
11	11
10	6
9	16
8	8
7	12
6	9
5	4
4	2
3	8
2	1
1	1
	100

TABLE IV.— (*concluded*).

Normal Apples, 1917. Number of seeds per apple.	Number of apples (frequencies).	*Aphis-Infested Apples, 1917.* Number of seeds per apple.	Number of apples (frequencies).
12	1	11	3
11	1	10	13
10	31	9	25
9	37	8	38
8	64	7	44
7	63	6	28
6	38	5	37
5	30	4	44
4	31	3	29
3	4	2	20
		1	14
	300	0	5
			300

SUMMARY.

Series.	Mean.	Standard deviation.	Coefficient of variability.	Range.	Number of observations.
Normal, 1916	10.05±.18	2.62±.13	26.11±1.33	4-16	100
Aphis, 1916	8.67±.22	3.25±.16	37.48±2.02	1-16	100
Difference	1.38±.28	....	11.37±2.42	3	....
Per cent increase*	—13.7	....	+43.5	+25.0	....
Normal, 1917	7.11±.07	1.83±.05	25.73±1.31	3-12	300
Aphis, 1917	5.57±.10	2.55±.07	45.76±2.60	0-11	300
Difference	1.54±.12	....	20.03±2.91	2	....
Per cent increase*	—21.7	....	+77.8	+22.2	....

* Normal apple constants taken as the base in calculating percentages.

It will be noted that the frequency distribution in each series is irregular, those of 1917 being more uniform than those of 1916. This is believed to be due to the paucity of the data. Even the use of three hundred observations in each series in 1917 did not suffice to remove all of the irregularities, so no attempt has been made to fit the frequency polygons with theoretical curves. From the summary of the data the following facts may be noted:

The average number of seeds per apple each year was greater in

normal than in aphis-infested fruits, and in each case the difference is significant.[1]

The coefficient of variability is more marked in aphis than in normal fruits both seasons, and the probable errors of the differences are sufficiently small to give confidence in the results. The difference in 1917 is the more marked.

While the range is usually considered one of the least important of the measures of variation, yet in these series several striking features may be noted. Each year the upper limits of the range are practically the same in injured and normal fruits, but in the former there is a considerable extension of the lower limits. This would indicate that the attacks by the rosy aphis prevented the dropping of fruits having few or no seeds, whereas fruits not infested and having few or no seeds dropped before picking time. The reasons for these differences are under investigation.

The percentage of increase (decrease if minus) of the constants for aphis apples over normal apples is noticeable in every instance.

Considering all the data this conclusion is reached: injuries by the aphis cause an extension of the range by permitting few seeded and sterile apples to remain on the tree, which has the effect of lowering the mean number of seeds per apple and thus increasing variability.

The variation in seeds during the several seasons also deserves mention in passing. The differences in the injured apples could be attributed to varying intensity of attacks by the aphis, but obviously this would not be true of normal fruits. Table V shows these differences between the two series.

TABLE V.— DIFFERENCES BETWEEN STATISTICAL CONSTANTS OF NUMBER OF SEEDS PER FRUIT OF NORMAL ROME APPLES FROM THE SAME ORCHARD DURING TWO SUCCESSIVE SEASONS.

Year.	Mean.	Standard deviation.	Coefficient of variability.	Range.
1916	10.05±.18	2.62±.13	26.11±1.33	4-16
1917	7.11±.07	1.83±.05	25.73±1.31	2-12
Difference	2.94±.19	....	0.38±1.87	2
Per cent decrease*	29.3	....	1.5	16.7

* 1916 constants taken as base in calculating percentages.

The apples of 1916 had a greater range and a higher mean number of seeds per apple than those of 1917, while the difference in variability was practically *nil*. Perhaps the most marked feature was the extension of the range to 16 seeds in 1916 over 12 seeds in 1917.

[1] Thruout this bulletin the usual rule of biometricians is followed and a difference is regarded as significant if it is at least three times its probable error.

As stated before, ten is considered about the normal seed content, and since this difference in the upper limits of the range occurred in aphis apples as well as in those not injured this variation is attributed to seasonal conditions.

WEIGHTS OF SEEDS IN NORMAL AND APHIS-INJURED FRUITS.

The nature of the distributions of the frequencies in the several series can be noted in the correlation tables (see Tables XII to XV inclusive). Table VI gives the statistical constants for the several series.

TABLE VI.— SUMMARY OF DATA OF WEIGHTS OF SEEDS IN NORMAL AND APHIS-INJURED ROME APPLES.
(Weights in milligrams.)

Series.	Mean.	Standard deviation.	Coefficient of variability.	Range.	Number of observations.
Normal, 1916	651.0± 9.7	144.1±6.9	22.14±1.11	270–1010	100
Aphis, 1916	375.0±12.8	189.8±9.1	50.61±2.97	6– 915	100
Difference	276.0±16.1	....	28.47±3.17	169	....
Per cent increase*.	—42.4	....	+128.6	+23.0	....
Normal, 1917	520.8± 7.3	108.4±5.2	20.82±1.04	302–753	100
Aphis, 1917	214.4± 8.3	123.1±5.9	57.43±3.54	0–599	100
Difference	306.4±11.1	....	36.61±3.69	148	....
Per cent increase	—58.8	....	+175.8	+32.8	....

* Normal apple constants taken as base in calculating percentages.

The following facts seem to be established by the summary of the data:

The average weight of seeds per apple each year was greater in normal than in injured fruits and in each case the difference is conspicuous.

The coefficients of variability both seasons are smaller in normal than in aphis-infested apples, and here again the errors are small.

Both the upper and lower limits of the range each season are higher in normal than in infested fruits.

The percentage of increase or decrease in the constants for aphis apples over normal apples are marked in every instance, those of the coefficients of variability being especially high.

In consideration of the foregoing data it may be concluded that, on the average, aphis-injured apples produce much lighter weight of seeds per apple than normal fruits, and that the variability in the weight of the seeds is greatly increased by the attacks of these insects. However, the upper limits of the range of weight of seeds in individual injured apples approach rather closely the maximum weight of seeds

found in normal apples. This is especially apparent in the series of 1916.

As will be shown later, there is high correlation between the weight of the seeds and the number of seeds per apple. It would be expected that the differences in weights of seeds in normal apples during the two seasons would be marked, for it has been shown above that considerable disparity existed in the number of seeds per apple in 1916 and in 1917. The data bearing on this point is brought together in Table VII.

TABLE VII.— DIFFERENCES BETWEEN STATISTICAL CONSTANTS FOR WEIGHT OF SEEDS PER APPLE IN NORMAL FRUIT DURING 1916 AND 1917.

(Weights in milligrams.)

Year.	Mean.	Standard deviation.	Coefficient of variability.	Range.	Number of observations.
1916	651.0± 9.7	144.1±6.9	22.14±1.11	270–1010	100
1917	520.8± 7.3	108.4±5.2	20.82±1.04	320– 753	100
Difference	130.2±12.1	....	1.32±1.52	307	....
Per cent decrease*	20.0	....	6.0	41.5	....

* 1916 constants taken as base in calculating percentages.

The average weight of seeds per apple and the range in weight of seeds in individual apples were greater in 1916 than in 1917, the percentage of decrease in these constants being marked. The difference between the two coefficients of variability is less than its probable error and therefore is not worthy of consideration.

WEIGHTS OF INDIVIDUAL FRUITS IN APHIS-INJURED AND NORMAL SERIES.

Several measurements could be employed to determine the amount of substance in an apple. In this study both volume and weight suggested themselves. It was tacitly assumed that the variation in specific gravity of the apples does not differ markedly between infested and normal fruits and that the determination of the weights would be a sufficient index of growth. Since the nature of the distribution of frequencies in the several series can be noted from the correlation tables (see Tables XVI to XIX inclusive) these have been omitted at this place because of considerations of space. Table VIII gives a summary of the constants for the several series.

An analysis of the data shows:

Each season the average normal apple was much heavier than the average aphis apple. The normal apples of 1916 averaged 2.6 times

the weight of the average aphis apple, and in 1917 they were 3.2 times heavier.

The variation as measured by the coefficient of variability is much larger in both aphis series than in the corresponding normal series.

TABLE VIII.— SUMMARY OF DATA OF WEIGHTS OF INDIVIDUAL ROME APPLES IN APHIS-INJURED AND NORMAL SERIES.

(Weights in grams.)

Series.	Mean.	Standard deviation.	Coefficient of variability.	Range.	Number of observations.
Normal, 1916...	131.0±1.1	16.84±0.80	12.86±0.62	83.0–200.6	100
Aphis, 1916.....	53.7±0.7	10.76±0.51	20.06±0.99	25.7– 85.2	100
Difference......	77.3±1.3	....	7.20±1.17	....	....
Per cent increase*.......	—59.0	....	+56.0	....	....
Normal, 1917...	134.4±1.6	23.31±1.11	17.34±0.85	92.3–190.3	100
Aphis, 1917.....	44.5±1.0	15.41±0.73	34.67±1.84	7.9– 81.8	100
Difference......	89.9±1.9	....	17.33±2.02	....	....
Per cent increase........	—66.9	....	+99.9	....	....

* Normal apple constants taken as base in calculating percentages.

The range in both series of normal apples is greater than the range of the aphis series of the same year. The lower limits of the range of normal weights for 1917 is slightly higher than the upper limits of the range of injured apples. In 1916 the upper limit of aphis apple weights slightly exceeded the lower limits of the range of weights of normal apples.

The per cent of increase or decrease in all constants is very noticeable. To summarize, it may be said that each season aphis apples were much lighter and markedly smaller than normal fruits, also that the variability was smaller in the uninjured apples.

Having found important differences in the number and weights of seeds in normal apples studied the two seasons, it is of interest to ascertain whether as marked differences occurred in the weights of the normal fruits during the same seasons, and in fact in the same series. The data are presented in Table IX.

It will be noted that the average weights of normal apples were nearly the same for the two seasons; in fact, the difference is less than twice the probable error and therefore might be attributed to mere fluctuations of random sampling. However, the variation as shown by the coefficient of variability and also by the standard deviation (since the means are practically the same) was considerably increased in 1917 over that of 1916. As attention has been

called to the marked differences in seed content and weights of the same between normal apples of these two years it is rather remarkable to find that the weights of the fruits should be so nearly the same. This would indicate that the relation between seeds and weights of fruits is not as marked as one might expect. The exact relation will be shown later in the study of correlation.

TABLE IX.— DIFFERENCES IN THE MEANS AND VARIATION OF WEIGHTS OF NORMAL ROME APPLES DURING 1916 AND 1917.

(Weights in grams.)

Year.	Mean.	Standard deviation.	Coefficient of variability.	Range.	Number of observations.
1916	131.0±1.1	16.84±0.80	12.86±0.62	83.0–200.6	100
1917	134.4±1.6	23.31±1.11	17.34±0.85	92.3–190.3	100
Difference	3.4±1.9	6.47±1.37	4.48±1.05	....	....
Per cent increase*	2.6	38.4	34.8	....	....

* 1916 constants taken as base in calculating percentages.

DEGREE OF CHANGE PRODUCED BY THE APHIS IN APPLE STRUCTURES.

Before leaving the studies regarding the influence of the insect on the several structures of the apple, which have just been considered, it may be well to add that the changes wrought by the aphis are not of the same intensity in the several parts of the apple. The data are set forth in Table X.

TABLE X.— THE PER CENT OF INCREASE OR DECREASE IN THE SEVERAL CONSTANTS DUE TO APHIS INJURY.

Structure.	Year.	Mean.	Coefficient of variability.	Range.
Seeds per apple, number	1916	—13.7	+43.5	+25.0
Seeds per apple, number	1917	—21.7	+77.8	+22.2
Seeds per apple, weight	1916	—42.4	+128.6	+23.0
Seeds per apple, weight	1917	—58.8	+175.8	+32.8
Fruit, weight	1916	—59.0	+56.0	....
Fruit, weight	1917	—66.9	+99.9	....

It will be noted that the least percentage decrease occurred in the means of number of seeds per apple and that there was a much greater decrease in the weights of the seeds, while the greatest decrease occurred in the weights of the fruits. It will also be observed that the

range was increased considerably in regard to weights and number of seeds, but the differences between the seasons are not great. In considering the ranges between weights of injured and normal apples of both seasons, we cannot calculate percentage differences as in each case the two ranges practically are separated so any calculation would be misleading. In the measure of the variation of the several structures we find that the injury has increased variability over normal fruit in every instance — that of weights of seeds being extremely large, that of number of seeds being least, while that of weights of fruits occupies a middle position. In other words, the insect does not affect all structures of the apple to the same degree. The smaller injured apples have not only been prevented in growing in size but have suffered a complete change in the relations of the several structures. Aphis apples are not merely small normal apples.

COMPARISON OF VARIABILITY IN THE SEVERAL APPLE STRUCTURES.

The variability of each structure or character has been treated separately. In order to compare the variation in the several structures the coefficients of variability in the four series are now considered together.

TABLE XI.— COEFFICIENTS OF VARIABILITY OF THE SEVERAL APPLE STRUCTURES.

Structure or character.	Normal, 1916.	Normal, 1917.	Aphis, 1916.	Aphis, 1917.
Number of seeds.....	26.11±1.33	25.73±1.31	37.48±2.02	45.76±2.60
Weight of seeds......	22.14±1.11	20.82±1.04	50.61±2.97	57.43±3.54
Weight of fruits.....	12.86±0.62	17.34±0.85	20.06±0.99	34.67±1.84

In normal apples the greatest variability occurred in the number of seeds per apple. The weights of the seeds were slightly less variable, showing that the weights of the seeds to a slight extent compensated for the change in the number of seeds. In these two characters the differences between the two coefficients of the two seasons are not marked. The smallest degree of variation occurred in the weights of fruits, and here the difference between the two seasons is significant.

In aphis-injured apples a marked increase in variability in every structure may be noted; weights of seeds having the highest coefficients, followed in turn by the number of seeds, while the lowest coefficients are in the weights of fruits, as was the case with the normal apples. The seed weight and number of seeds do not move together as closely in the aphis apples as in normal fruit.

SOME RELATIONSHIPS BETWEEN SEED AND FRUIT OF NORMAL AND APHIS APPLES.

Since the nature of the distributions as regards variation have been studied it was desired to learn how the several structures or characters were interrelated. In other words, is variation in one structure connected by some causative relation to variation in another structure? In order to answer this question there were considered the relations existing between the several structures or characters by means of a study of correlation and regression.

THE CORRELATION OF APHIS-INJURED AND NORMAL ROME APPLES AS REGARDS WEIGHT AND SEED DEVELOPMENT.

Having discussed the variation both of normal apples and of those injured by rosy aphis when the several structures or characters were treated singly, attention is now directed to the correlation of these structures and characters. Two problems, each consisting of two distinct phases (since the behavior of both normal and abnormal fruits are involved) present themselves for consideration, and they are as follows:

1. To determine the relation existing between the number of seeds in an apple and their weight. Some concrete questions suggested by this inquiry are: Do apples of high seed content produce a greater weight of seeds than those of low seed content, or do sixteen-seeded apples produce smaller seeds than six-seeded apples in the same series, and is the difference in size sufficient to equalize the total weight of seeds per apple, at least to a considerable extent? If a high degree of correlation exists between the number and weights of seeds in all the series (and it will be necessary to mention that this has been found to be true) weights of seeds may be used instead of number of seeds in the study of the relation between seed and pulp development, as this permits of a more flexible grouping.
2. To ascertain the relation of seeds to fruit development. Considering the data at hand, is a high seed weight related to large size of fruit in both normal and aphis apples?

In a manner some of these questions have been answered previously, but more comprehensive as well as more exact relationships can be ascertained by the study of correlations and their resulting regression equations.

The first correlations are those of weight of seeds and number of seeds per apple in the four series which are shown in Tables XII to XV inclusive. The second series of correlations are those of weight of seeds and weight of fruits, as given in Tables XVI to XIX inclusive.

TABLE XII. CORRELATION BETWEEN WEIGHT OF SEEDS AND NUMBER OF SEEDS PER APPLE.

NORMAL APPLES, 1916.

Mid-values.	Seed Weight in Milligrams.																Total.
	275	325	375	425	475	525	575	625	675	725	775	825	875	925	975	1025	
Number of seeds. 4	1																1
5			2														2
6				4	2												6
7				1	4	3											8
8						3	7	3									13
9					1	1	5	5									12
10						2	1	7	5	4							19
11							1	1	2	4	1	1					10
12									2	4	3	1					10
13											2	2	1	1			6
14											2	2	4				8
15													2			1	3
16													1		1		2
Total	1	0	2	5	7	9	14	16	9	12	8	6	8	1	1	1	100

TABLE XIII.— CORRELATION BETWEEN WEIGHT OF SEEDS AND NUMBER OF SEEDS PER APPLE.

APHIS APPLES, 1916.

Mid-values.	Seed Weight in Milligrams																Total
	30	90	150	210	270	330	390	450	510	570	630	690	750	810	870	930	
Number of seeds. 1	1																1
2			1														1
3	1	2	3	2													8
4				2													2
5				1	1	2											4
6	1			1	1	2	4										9
7	1			3	3	3	1	1									12
8					2		2	2	2								8
9	2		1	2	2	1	2	3	2	1							16
10					1		1	2	2								6
11	1			1			1	2	2	1	3						11
12		1						4	1		1	2					9
13									1	3	1	2	2				9
14									1		1						2
15																	0
16							1									1	2
Total	7	3	5	12	10	8	12	14	11	5	6	4	2	0	0	1	100

TABLE XIV.— CORRELATION BETWEEN WEIGHT OF SEEDS AND NUMBER OF SEEDS PER APPLE.

NORMAL APPLES, 1917.

Mid-values.	Seed Weight in Milligrams.												Total
	300	340	380	420	460	500	540	580	620	660	700	740	
Number of seeds. 4	3	4				1		1					9
5		1	1	2									4
6	1		3	2	5	1	1	1					14
7		1			4	7	6	1		2			21
8			1		2	7	3	3	6	2			24
9		1			1		4	1	4	1	1	2	15
10					1	1	1	2	1	2	5		13
Total	4	7	5	4	13	17	15	9	11	7	6	2	100

TABLE XV.— CORRELATION BETWEEN WEIGHT OF SEEDS AND NUMBER OF SEEDS PER APPLE.

APHIS APPLES, 1917.

Mid-values.	Seed Weight in Milligrams															Total
	20	60	100	140	180	220	260	300	340	380	420	460	500	540	580	
Number of seeds. 0	2															2
1	1	1														2
2	1	2	1													4
3			3	1	1	1										6
4	1	2	3	5	2	2	1									16
5	1	2	1		4	2	1									11
6		1	1		3	4	1	2	1							13
7	1			3	4	2	2	1	2							15
8					2	2	1	3	2	4						14
9					1	2			3		3			2		11
10							1		1	1		1			1	5
11									1							1
Total	7	8	9	9	17	15	7	6	10	5	3	1	0	2	1	100

TABLE XVI.— CORRELATION BETWEEN WEIGHT OF SEEDS AND WEIGHT OF FRUIT.

NORMAL APPLES, 1916.

Mid-values.	Seed Weight in Milligrams.																Total.
	275	325	375	425	475	525	575	625	675	725	775	825	875	925	975	1025	
Fruit weight in grams. 85													1				1
95	1																1
105				1		1	3	1									6
115					1	2	3	2	2	1	2	1	1				15
125				1	2	3	1	6	2	3	2	1	2		1		24
135			1	2	1	2	7	3	3	5	3	1	3			1	32
145			1		3			3	2	1		1	1				12
155				1				1		1	1	1					5
165																	0
175										1		1		1			3
185																	0
195																	0
205						1											1
Total	1	0	2	5	7	9	14	16	9	12	8	6	8	1	1	1	100

PLATE XXV.— THE ROME APPLE AND A LONGITUDINAL SECTION.

PLATE XXVI.—ROME APPLES INJURED BY THE ROSY APHIS.

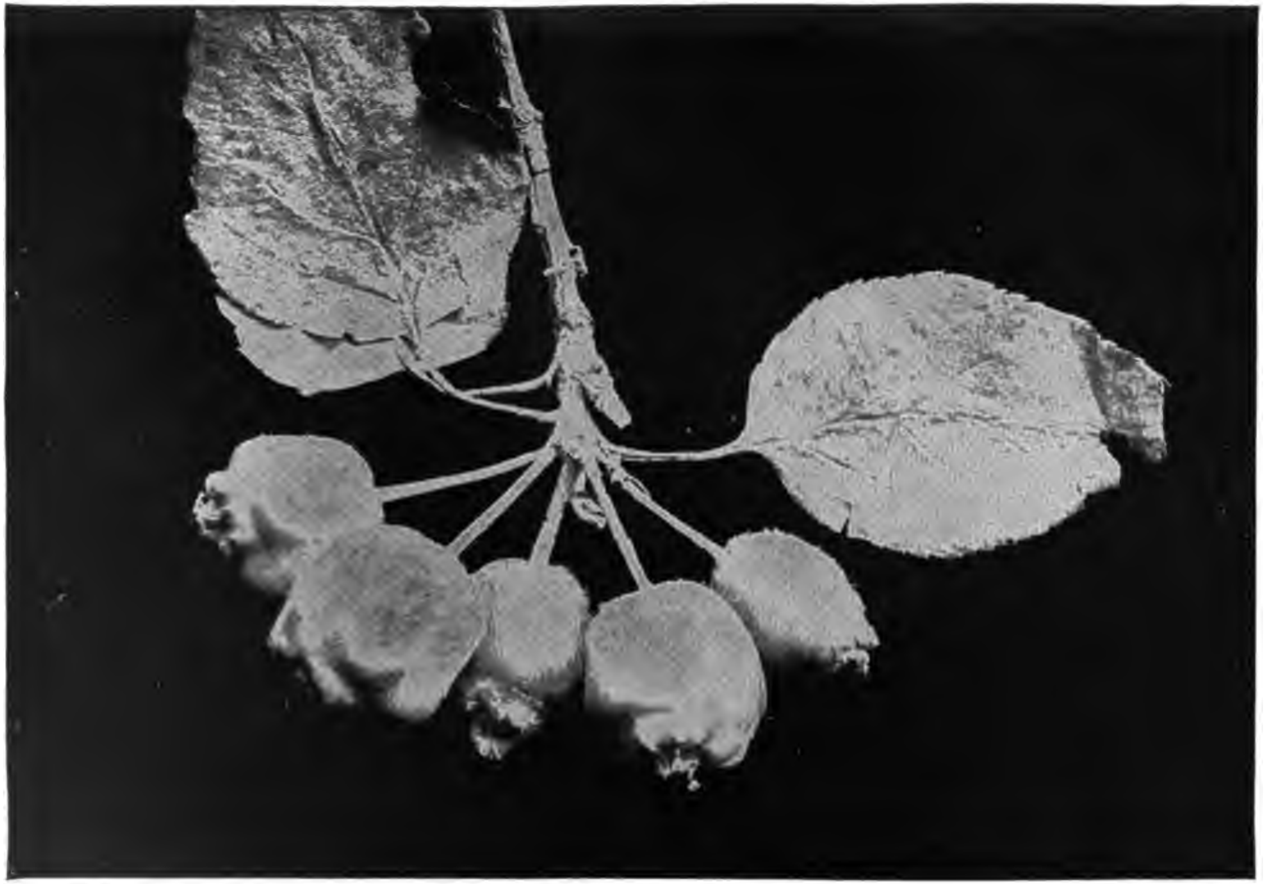


PLATE XXVII.— DEFOLIATION AND CLUSTER OF SMALL DEFORMED APPLES DUE TO ROSY APHIS.

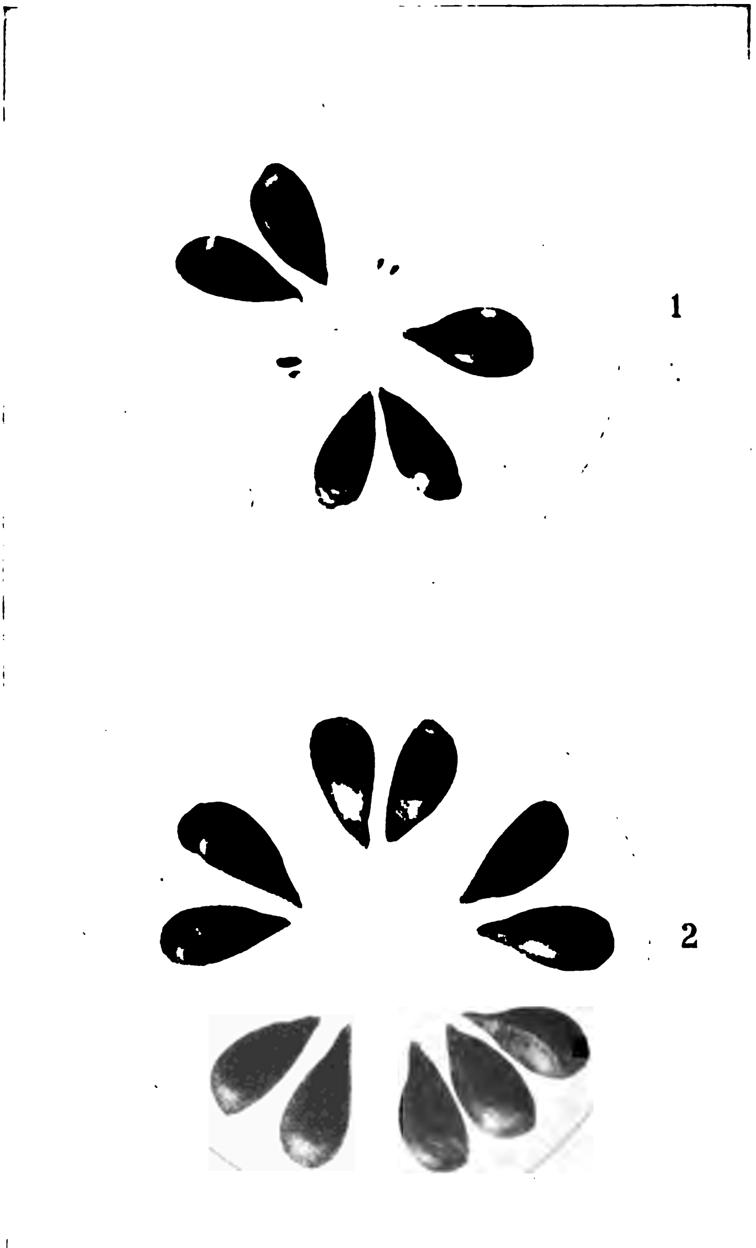


Plate XXVIII.— Seeds from Two Normal Rome Apples.
(Magnification the same as in Plate XXIX.)

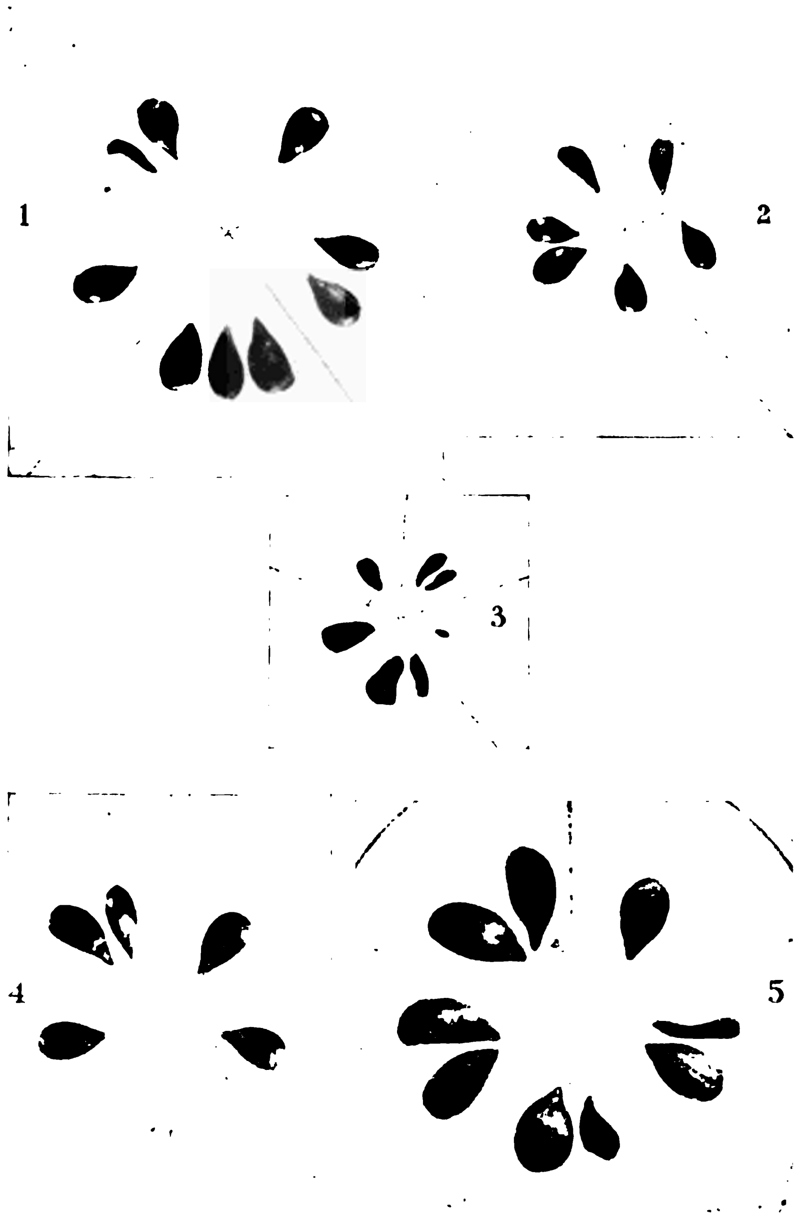
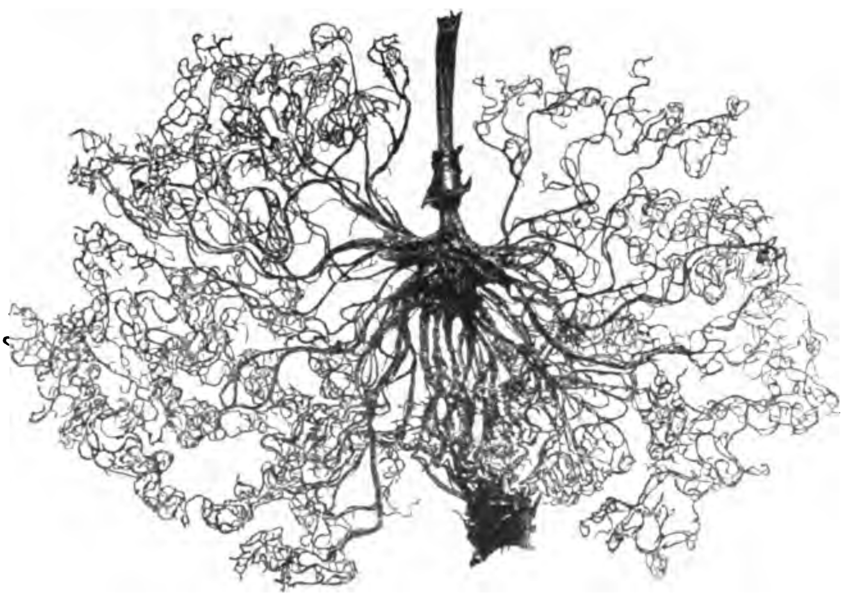


Plate XXIX.— Seeds from Five Rome Apples Injured by the Rosy Aphis.
(Magnification the same as in Plate XXVIII.)

PLATE XXX.— SEEDS FROM SEVEN TYPICAL FRUITS.
1, Aphis infested apples; 2, normal Rome apples (with same magnification.)

1



2

PLATE XXXI.— FIBRO-VASCULAR STRUCTURES OF ROME APPLES.
1, Aphis-injured fruit; 2, Normal fruit. (Natural size.)

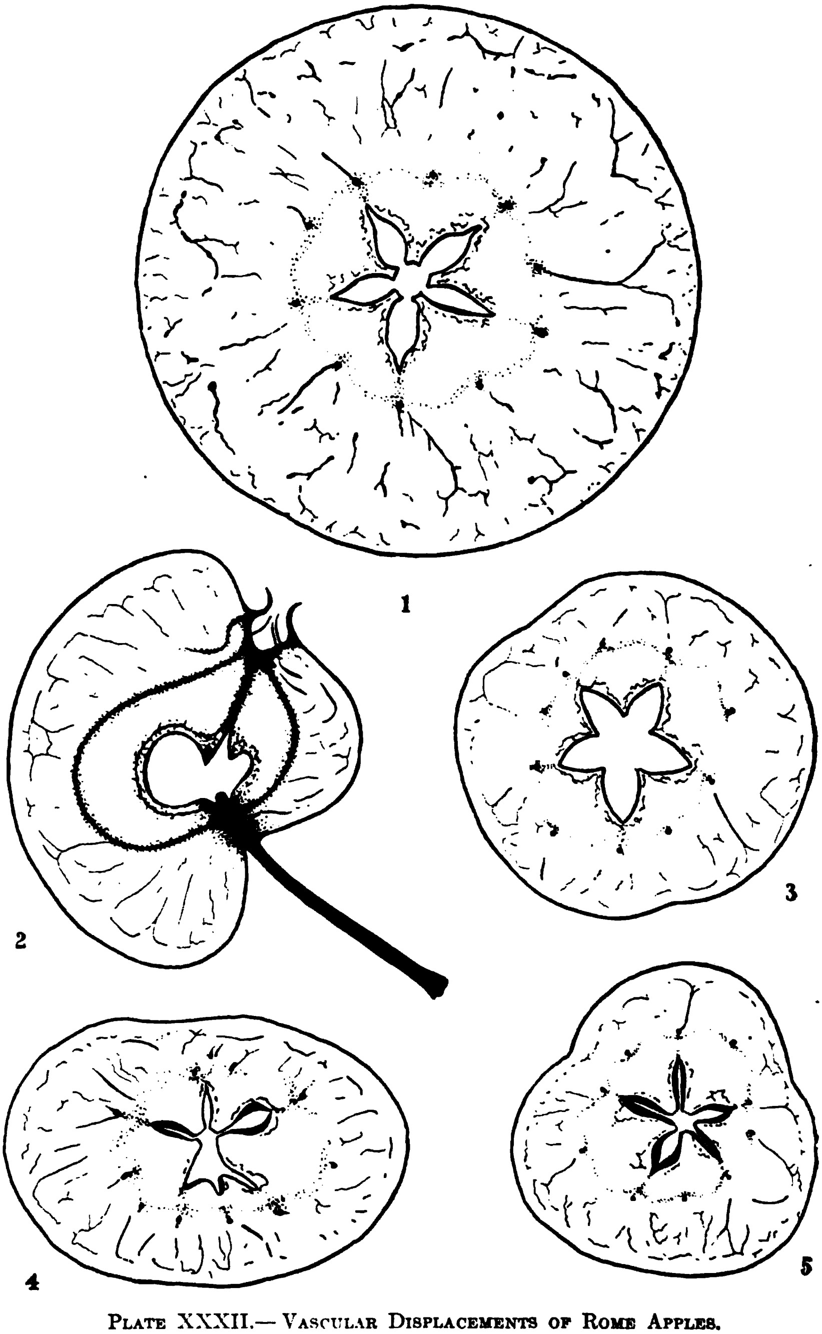


PLATE XXXII.— VASCULAR DISPLACEMENTS OF ROME APPLES.
1, Section of normal fruit; 2, 3, 4 and 5, Sections of injured fruits.

TABLE XVII.—CORRELATION BETWEEN WEIGHT OF SEEDS AND WEIGHT OF FRUIT.
APHIS APPLES, 1916.

Mid-values	Seed Weight in Milligrams.																Total.
Fruit weight in grams.	30	90	150	210	270	330	390	450	510	570	630	690	750	810	870	930	
27.5	1	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	2
32.5	2	1	...	1	...	...	...	...	...	...	...	...	...	...	...	...	4
37.5	...	1	1	...	...	...	...	...	1	...	...	...	...	...	...	...	3
42.5	1	...	1	3	3	...	3	1	...	...	...	1	...	...	...	...	13
47.5	...	...	...	2	1	2	...	1	3	...	1	...	...	...	...	...	10
52.5	2	...	1	2	4	2	1	3	1	2	2	2	...	...	...	...	22
57.5	1	...	1	1	1	2	2	5	3	3	2	...	...	...	...	1	22
62.5	...	...	1	1	...	1	2	3	1	...	...	1	1	...	...	...	11
67.5	...	1	...	...	...	1	2	1	1	...	...	...	...	...	...	...	6
72.5	...	...	...	2	...	...	2	...	...	...	...	...	1	...	...	...	5
77.5	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	1
82.5	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0
87.5	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	1
Total	7	3	5	12	10	8	12	14	11	5	6	4	2	0	0	1	100

TABLE XVIII.—CORRELATION BETWEEN WEIGHT OF SEEDS AND WEIGHT OF FRUIT.
NORMAL APPLES, 1917.

Mid-values.	Seed Weight in Milligrams.												Total.
Fruit weight in grams.	300	340	380	420	460	500	540	580	620	660	700	740	
95	...	...	...	...	...	2	1	...	...	1	...	...	4
105	1	2	1	...	4	4	...	...	1	1	...	...	14
115	1	1	3	...	5	...	1	...	2	...	2	...	15
125	1	...	...	1	...	3	1	2	1	...	1	...	10
135	1	1	1	...	2	1	5	3	2	1	1	1	19
145	...	1	...	1	1	1	2	3	1	1	1	...	12
155	...	1	...	1	1	1	4	...	1	1	...	...	10
165	...	1	...	1	...	2	...	1	2	1	1	...	9
175	...	...	...	...	...	1	...	...	...	1	...	1	3
185	...	...	...	...	...	1	1	...	1	...	...	...	3
195	...	...	...	...	...	1	...	...	...	...	...	...	1
Total	4	7	5	4	13	17	15	9	11	7	6	2	100

TABLE XIX.—CORRELATION BETWEEN WEIGHT OF SEEDS AND WEIGHT OF FRUIT.
APHIS APPLES, 1917.

Mid-values.	Seed Weight in Milligrams															Total.
Fruit weight in grams.	20	60	100	140	180	220	260	300	340	380	420	460	500	540	580	
9	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1
15	...	1	1	...	...	...	...	...	...	...	...	...	...	...	...	2
21	2	1	2	1	1	1	...	...	...	...	...	...	...	...	...	8
27	1	1	2	...	5	...	...	...	...	...	...	...	...	...	...	9
33	...	...	2	2	1	2	1	...	1	...	...	...	...	...	...	9
39	1	1	...	4	4	3	1	1	1	1	...	...	...	...	...	17
45	1	1	1	...	1	2	1	3	...	...	1	...	...	1	...	12
51	...	1	1	1	...	5	1	...	3	2	1	...	...	...	...	15
57	1	1	...	...	1	...	1	1	...	1	1	...	...	...	...	7
63	...	1	...	1	2	1	2	...	3	1	...	1	...	...	1	13
69	...	...	...	...	2	...	...	1	...	...	...	...	...	1	...	4
75	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	1
81	...	...	...	...	...	1	...	...	1	...	...	...	...	...	...	2
Total	7	8	9	9	17	15	7	6	10	5	3	1	0	2	1	100

The coefficients of correlation and the coefficients of regression calculated from Tables XII to XIX are shown in Table XX.

TABLE XX.—COEFFICIENTS OF CORRELATION AND COEFFICIENTS OF REGRESSION. APPLE STRUCTURES AND CHARACTER.

Structure or character.	Series.	Coefficient of correlation.	Coefficient of regression.	Difference of regression coefficients. (Normal and aphis.)
Seed weight on number.	Normal, 1916	.945+.007	51.88+0.40	....
Seed weight on number.	Aphis, 1916	.705±.034	41.20±1.98	10.68±2.02
Seed weight on number.	Normal, 1917	.777±.027	49.97±1.84	....
Seed weight on number.	Aphis, 1917	.759±.029	38.82±1.46	10.97±2.35
Fruit weight on seed weight..............	Normal, 1916	.119±.066	.0139±.0078	....
Fruit weight on seed weight..............	Aphis, 1916	.359±.059	.0204±.0033	.0065±.0085
Fruit weight on seed weight..............	Normal, 1917	.225±.064	.0484±.0138	....
Fruit weight on seed weight..............	Aphis, 1917	.493±.051	.0619±.0064	.0135±.0152

The discussion of these coefficients is deferred until the regression equations and the diagrams representing them have been considered because comparisons and deductions can better be made by having more of the calculated data in mind.

REGRESSION AND THE DETERMINATION OF LAWS DESCRIBING RELATED PHENOMENA.

One of the objects of studies of correlated variability is to enable the investigator to predict the occurrence of attributes of characters or structures from a knowledge of attributes of related structures or characters. If in a correlation table the means of each series of arrays be plotted against the mid-values of the related characters for the same arrays, using the values of one series as ordinates and the other as abscissas, the points thus obtained usually lie nearly on a straight line or, if scattered, are generally so distributed that a straight line will fit the points as well as a more complicated curve.[1]

By determining the equation of a straight line or of any other curve we obtain an expression for the attributes of one of the structures or characters in terms of the attributes of the other structure or character. This equation is a mathematical expression of the law describing the relations of one attribute with another. This law is determined by the principle of regression and is known as the regression equation. When the plotted points lie on a straight line

[1] Yule, G. U. An Introduction to the Theory of Statistics, 2d Ed., London, 1912, p. 169.

the regression is " linear," but if the points lie on a more complex curve the regression is " non-linear " and the correlation is " skew ".

LINEAR REGRESSION.

There are mathematical tests of linearity, but since the number of observations in each of the tables is few and as the diagrams exhibit clearly that the regressions in the eight tables are linear it is not considered necessary to make the calculations for these tests.

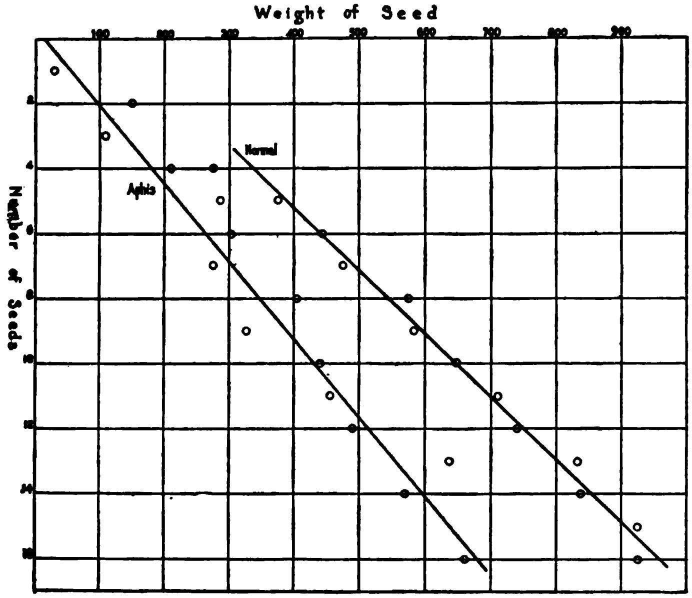


FIG. 10.— REGRESSION OF WEIGHT OF SEEDS ON NUMBER OF SEEDS PER APPLE, 1916 SERIES.

In order to facilitate examination the two regressions to be compared are placed in the same figure.

The following regression equations have been calculated:

Normal apples, 1916.	*W. S. = 51.88 N + 129.61.	W. F. = .01392 W. S. + 121.94.
Aphis apples, 1916.	W. S. = 41.20 N + 17.80.	W. F. = .02039 W. S. + 46.00.
Normal apples, 1917.	W. S. = 49.97 N + 149.06.	W. F. = .04836 W. S. + 109.21.
Aphis apples, 1917.	W. S. = 38.82 N — 19.30.	W. F. = .06169 W. S. + 31.19.

* The following abbreviations have been used in the several equations: N, number of seeds per apple; W. S., weight of seeds per apple, and W. F., weight of fruits.

It is obvious from Figures 10 and 11 that the regression of seed weight on number of seeds per apple in every series is strictly linear. In the series of aphis apples each year the regression of fruit weight on seed weight is linear (Figs 12 and 13), but in the normal series both seasons the regressions of fruit weight on seed weight, while linear, show a scattering of the points, and near the ends of the ranges several means — in every case based on a few observations — depart markedly from the regression lines. However, the straight line curve shows the trend of the regression, and that is about all that can be expected with the limited number of observations.

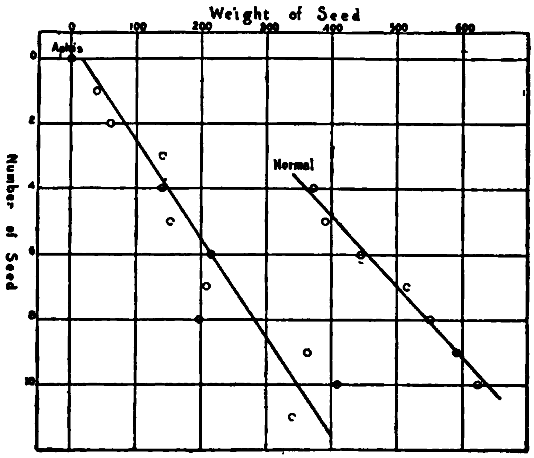


FIG. 11.— REGRESSION OF WEIGHT OF SEEDS ON NUMBER OF SEEDS PER APPLE, 1917 SERIES.

COMPARISON OF THE CORRELATION COEFFICIENTS AND REGRESSION OF STRUCTURES OR CHARACTERS IN INJURED AND NORMAL FRUITS.

The purpose of this bulletin, as mentioned before, is to compare the several structures in normal apples and those injured by the rosy aphis. Having calculated the various constants and determined the laws of regression of the several structures and characters, we are in a position to make comparisons.

RELATION BETWEEN THE WEIGHT OF SEEDS AND NUMBER OF SEEDS PER APPLE.

From Table XX it will be noted that the correlation coefficient for these characters in every series is positive and has a high value. This means that on the average high seed content gives a high weight of seeds. Stated otherwise, the size of seeds, for example in 16-seeded apples, have not been so reduced as to weigh but slightly more than those of, say, 15-seeded or 12-seeded apples, but

the higher the number of seeds per apple the greater the weight of the seeds. There is a slight reduction in the size of the individual seeds as the number per apple is increased, and this is emphasized by the fact that the coefficient of correlation in only one instance is very close to 1.000.

When we compare the two series for each season we find that there are differences each season in the coefficients of correlation and that

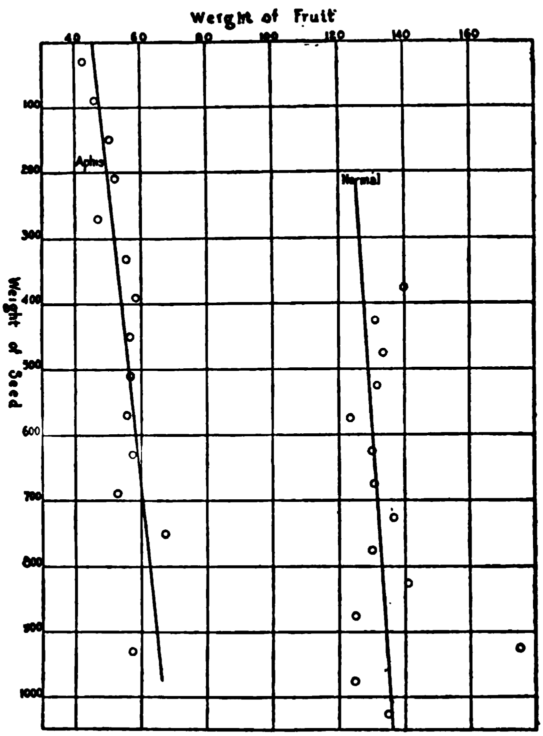


FIG. 12.— REGRESSION OF WEIGHT OF FRUIT ON WEIGHT OF SEEDS PER APPLE, 1916 SERIES.

in 1916 normal apples gave the higher coefficient, while in 1917 aphis-injured apples showed the higher correlation.

A glance at Figures 10 and 11 shows that for the same year the regression lines are nearly parallel; however, in both series the lines show a slight tendency to converge. A review of the coefficients of regression for these seasons exhibits differences of 10.68±2.02 and 10.97±2.35, which is more than three times their probable errors, and therefore significant. This means that the aphids de-

creased the mean number of seeds and also the mean weight of those seeds, and that the proportionate deviation in weight of seeds from the mean as one passes from the apples of low seed content to those of high seed content was not the same as in normal apples.

RELATION BETWEEN THE WEIGHT OF FRUITS AND THE WEIGHT OF SEEDS.

The coefficients of correlation between weight of fruits and weight of seeds in the four series are all positive (see Table XX) and both series of normal apples have low coefficients. The aphis-injured

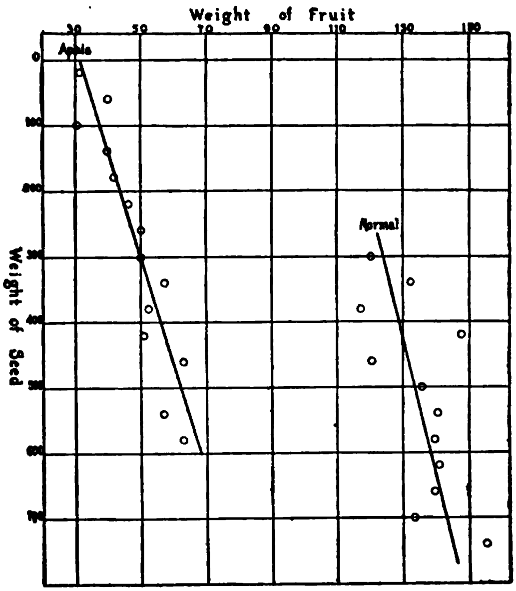


FIG. 13.— REGRESSION OF WEIGHT OF FRUIT ON WEIGHT OF SEEDS PER APPLE, 1917 SERIES.

apples show higher correlation than normal apples, but these coefficients are not very high. The upshot of all this data is that there appears to be a closer relation between fruit weight and seed weight in small apples than in large ones. This suggests that the coefficient of correlation continues to grow smaller as the sizes of the fruits increase, and that in the normal apples of the two series the fruit had reached such a size that the relationship between pulp and seed was very slight. The difference in weight of normal fruits for the two seasons is insignificant and the difference between the two coefficients of correlation for normal apples is not marked when the probable errors are considered. On the whole the relation between

seed weight and fruit weight is not as marked as has generally been believed.

A comparison of the coefficients of regression (see Table XX) shows that the differences between those of aphis-injured and normal fruits each season are less than their probable errors. This means that the regression lines are practically parallel. Stated otherwise, aphis injury does not change the degree of deviation from the mean over that found in normal apples, altho the mean weight of fruits and the mean weight of seeds has been markedly changed.

INFLUENCE OF APHIS ON FIBRO-VASCULAR SYSTEM.

There is an essential relationship between the development of the conducting vessels, the growth of seeds and the fleshy receptacle. The abnormal conditions of aphis apples with respect to size and shape and the number and weight of their seeds as previously described raise the question if such irregularities are also accompanied by disturbances of the fibro-vascular bundles.

The vascular system of the apple has been the subject of careful investigations by McAlpine,[1] of Australia, and Kraus,[2] of Oregon. In order that certain effects on the structures as a result of the work of the aphis may be more clearly explained, it is desired to allude to some facts revealed by these studies. The latter writer recognizes five general regions of the conducting system, certain of which are described with great detail. According to him, if an " apple is cut about the middle at right angles to the axial diameter, ten green spots may be observed arranged in a circle about midway between the center and the skin, following closely the so-called core line, which marks the boundary line between the modified pith and cortex. These ten bundles are arranged in two cycles: those of the outer cycle are located opposite the dorsal sutures of the carpels and are farther from the vertical axis of the fruit, while those of the inner cycle alternate with the carpels." From their position they are, according to McAlpine,[3] evidently " developed in connection with the five carpels, for there is one strand opposite each of the seed cavities and another in an intermediate position, making ten in all. Strong corroborative evidence is afforded that the bundles are developed in connection with the carpels by the fact that when the abnormal number of six carpels occur there are twelve bundles instead of ten, and when there are four carpels there are only eight strands. In a longitudinal median section of the apple each of these

[1] McAlpine, D., The Fibro-Vascular System of the Apple (Pome) and its Functions, Proc. Linn. Soc., N. S. Wales, Vol. XXXVI, Pt. 4, pp. 613–625, 1912.

[2] Kraus, E. J., Gross Vascular Anatomy of the Apple, Oregon Experiment Station, Bul. No. 138, 1916.

[3] McAlpine, D., Bitter Pit Investigation, First Progress Report, pp. 35–36, 1911–12, Melbourne, Australia.

ten vascular bundles is seen to give rise to branches, which in turn branch again and so on, mostly towards the outside, altho there are several branches on the inside. From each of these ten strands, just as they are leaving the stalk, branches are given to the outer and inner face of the seed cavity, so that the seeds are well supplied." He also states that "the main strands are associated with the flesh, and the diverging branches towards the outside do not divide much until they approach the skin, where they form a perfect network. This vascular net envelopes the flesh about one-quarter of an inch or less from the surface, and this wonderful and unsuspected structure not only unites the entire system of vessels but gives rise to the innumerable plume-like branches which reach even to the skin. These arise from the boundaries of each mesh of the net, and they divide and subdivide in such a luxuriant manner that the ultimate branches interlace and intertwine so as to form a seemingly continuous layer of conducting tissue beneath the skin."

Following the methods used by the foregoing writers, the pulp was removed from a number of normal Rome apples, and the different vascular elements with their characteristic branching are shown in Plate XXXI, fig. 2. Aphis apples of the same variety, similarly treated, exhibited a vascular network as illustrated in Plate XXXI, fig. 1. The most marked differences between the vascular systems of the normal and aphis-infested apples, which are clearly apparent when the soft parts are removed, are that the primary strands of the former are generally thicker and longer, while the diverging bundles that are distributed thruout the peripheral areas of the fruits form a more dense network of plume-like branchlets. In cross sections of normal apples the primary bundles present quite a uniform appearance as to size and the extent of the interspace between each other and distance from the vertical axis. Aphis apples that displayed considerable distortion on one side generally showed vascular displacement with respect to the distance of primary bundles from vertical axis and the amount of space between the individual strands. (Plate XXXII.) Even with severely malformed fruits no reduction was observed in the number of the primary vascular structures. As previ usly pointed out, the vascular elements on the affected side of the apple may show considerable displacement and suppression in development, while the ultimate branchlets seemed to resemble more those of a half-grown apple in that they do not display the dense plume-like appearance so characteristic of a normal-ripened fruit.

MALFORMATIONS OF APPLES DUE TO OTHER CAUSES.

It has been shown that the most noticeable effects of the rosy aphis on apples are arrestment of growth, distortions of the calyx and stem basins, and retention to an abnormal extent of seedless

fruits on trees at time of harvesting. Apples displaying these malformations present a characteristic appearance and are readily recognized by the ordinary observer. In this connection mention should be made of the fact that, while the larger proportion of the apples produced by a tree are within certain limits identical in appearance and therefore classified as normal or typical for the variety, still there is a percentage of the fruits that assume a different form from the general mass. The deviations from typical apples are not usually very conspicuous and consist chiefly of irreg-

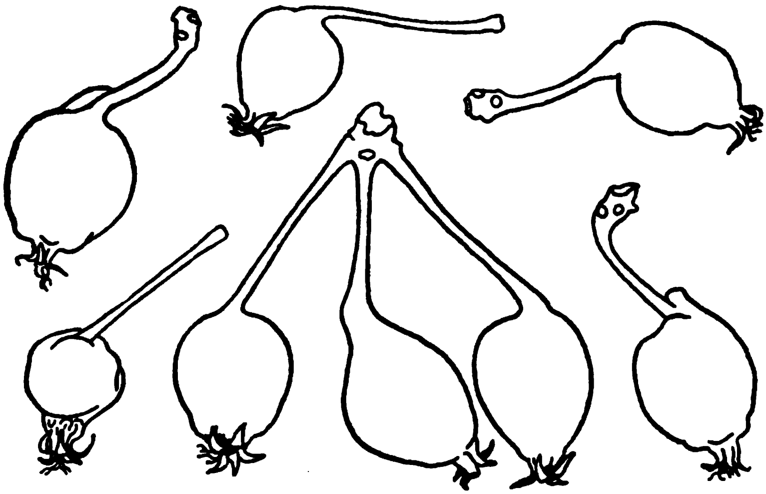


FIG. 14.— ATYPICAL FORMS OF THE ROME APPLE.

ular developments in form and structural arrangements, known to students of pomology as lob-sidedness, clubbed stem and oblique stem. The abnormalities of this character that are most common with the Rome apple are illustrated in Fig. 14. These defects occur independent of the work of the rosy aphis, but attacks of such fruits by the insect tend to aggravate the distortions. Likewise the lack of color of aphis apples may be accounted for in part by the preference of the pest for the shaded, lower and interior areas of the trees, where apples are normally not so highly colored as those more exposed to sunlight.

COMPARISON OF METHODS FOR COMPUTING DAILY MEAN TEMPERATURES: EFFECT OF DISCREPANCIES UPON INVESTIGATIONS OF CLIMATOLOGISTS AND BIOLOGISTS.*

F. Z. HARTZELL.

SUMMARY.

The daily mean temperature is the thermal-time unit in most general use among climatologists and ecological workers in botany and zoology. This is the average of hourly-Fahrenheit-degree units for the twenty-four hours of the day. Since its exact determination necessitates the use of accurate thermographs which require careful manipulation and correction, approximate methods of determining the daily mean temperature are common. Usually the average of the highest and lowest temperatures for a day, determined by maximum and minimum thermometers read at some convenient hour, is employed.

Since the degree of heat generally varies considerably during a day, the temperature curves are irregular, and the average of the highest and lowest values does not give the true mean temperature. True daily means may be secured by the summation of the average hourly temperatures, divided by twenty-four; or with the drum type of thermograph the summation may be secured mechanically by integrating the temperature curve with a planimeter and dividing the result by twenty-four. This true mean is designated the thermograph average, while the approximate mean is known by the hour at which the observations are recorded; viz., midnight or 12 P. M., 8 P. M. and 5 P. M. averages.

The difference between a true mean and an approximate mean is designated a discrepancy and may be positive, if greater than the thermograph average, or negative, if less.

The amount of the discrepancies introduced by approximate means for the several hours of observation were investigated at Fredonia, N. Y., for every day of 1916.

On the basis of a year's record, in general, it was found that: (1) small discrepancies occur more frequently than large ones; (2) within the limits of the times of observation used in this study, the time of observation determines the probability of large discrepancies, the five o'clock averages producing many more large values than averages from observations made later in the day; (3) positive

* Reprint of Technical Bulletin No. 68, June, 1919.

discrepancies are of more frequent occurrence than negative ones, thus causing the approximate annual mean temperature to be too high, and these differences are the greater the earlier the hour of observation; (4) no method of using maximum and minimum temperatures is as exact as that based on the summation or integration of the thermograph curve.

The discrepancies in the annual mean temperature are: for the five o'clock record, 1.21 degrees; for the eight o'clock averages, .58 degree; and for the midnight averages, .20 degree. Thus, from a practical viewpoint, averages of daily maximum and minimum temperatures, when made not earlier than 8 P. M., affect the annual mean temperature so slightly that the differences are negligible, but averages of observations earlier than this hour introduce differences that may be important.

The standard deviations of the several distributions were: midnight, 1.58 degrees; eight o'clock, 2.33 degrees; and five o'clock, 3.09 degrees. This shows that, of the approximate averages, those of midnight were the most accurate, the five o'clock record was the least accurate, while that of eight o'clock occupies a middle position.

A comparison of the range of discrepancy, as given by the different records, is as follows: midnight, —5.0 to 5.3; 8 o'clock, —10.0 to 10.1; and five o'clock, —10.5 to 14.6.

The greatest daily discrepancies occurred during the winter months, and the least during the summer months. July showed the smallest deviations from the thermograph average.

Considering monthly mean temperatures, the five o'clock record gave positive average discrepancies every month, and, while an extreme discrepancy of two degrees occurred one month, the usual value was between one and two degrees.

Altho the eight o'clock averages produced average monthly discrepancies which were above one degree during three of the months, usually they were nearer one-half degree too high.

The midnight average seldom gave average discrepancies of more than one-half degree for any month.

For meteorological purposes, the monthly differences were not excessive when the hour of observation was not earlier than eight P. M., but for biological purposes it is advisable to use the thermograph averages. If approximate averages are employed, the same hour of observation should be used if the data are expected to be comparable.

During 1916 the number of days having discrepancies of five degrees or more, either positive or negative, were: midnight record, 2; eight o'clock, 16; and five o'clock average, 39.

The probable discrepancy for a single day for 1916 was: ±1.07 degrees for the midnight average; ±1.57 degrees for the eight o'clock; and ±2.09 degrees for the five o'clock average.

Owing to the extreme variation in the discrepancies from day to day, the use of the shorter method of computing means may introduce errors which will mask relationships between thermal influence and biological activity.

For the comparison of daily changes in temperature, thermograph means alone give precise results.

The summation of effective temperature or of temperature coefficients for the study of thermal influence in botany or zoology should be calculated from thermograph averages, since approximate means introduce rather large errors, especially during the spring months.

To secure accurate averages of all kinds, the ecological worker should rely upon thermograph records which have been checked, and, if necessary, corrected from readings of maximum and minimum thermometers of precision.

INTRODUCTION.

The activities of both plants and animals are greatly influenced by the amount of heat affecting their environment during a given interval of time. In biological investigations, the thermal factor is very important, and for this reason temperature data are carefully recorded, the ultimate object being to interpret the influence of the several climatic components on the rate of development of the subjects under study. Inasmuch as heat plays so extensive a role in the physiology of plants and animals, it is essential that temperature records accurately indicate the amount of heat present during the period of observation.

There are several methods of arriving at an estimate of this value, the use of the daily mean temperature being the most common. In calculating this mean by the methods most commonly in use, certain deviations from the true value have been found to occur. The sources and extent of the discrepancies that enter into the several methods of calculating mean temperatures, together with the errors which they introduce in summations and differences between the temperature of succeeding days, were investigated and form the subject matter of this bulletin.

The manuscript has been read by Mr. C. A. Donnel, Meteorologist, U. S. Weather Bureau, for which courtesy and for references to the literature we are under obligation.

METHODS OF INDICATING THE AMOUNT OF HEAT PRESENT DURING A DAY.

Temperature represents a static condition of heat. The ordinary thermometer shows the degree of heat present at the time the reading is made. It is a linear quantity — a single dimension, so to speak — and its value, for practical purposes, would be lessened if this were

the only unit possible. It is essential that the duration of time as well as the temperature be considered together if climatological[1] comparisons are to have any value. In other words we must pass to a temperature-time concept. This is no new idea, for we are familiar with the expressions "daily mean temperature," "monthly mean temperature," etc., but the discussion of temperatures is often of such a nature as to lead the reader to infer that the writer was considering temperature without relation to time. While time and temperature each are linear quantities, when combined they represent *second dimension quantities* and, as such, may be represented by surfaces. The familiar temperature curves, traced automatically by a thermograph, may be cited as illustrations.

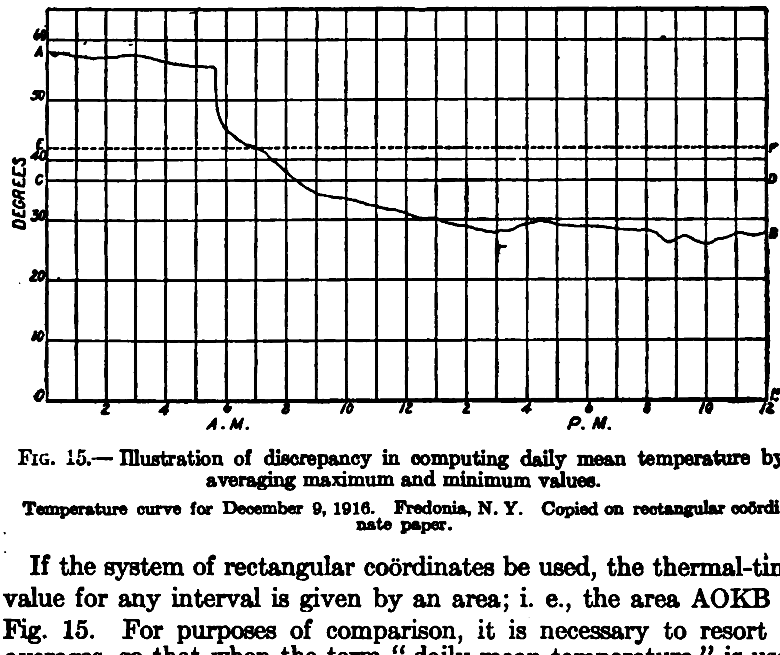


FIG. 15.— Illustration of discrepancy in computing daily mean temperature by averaging maximum and minimum values.

Temperature curve for December 9, 1916. Fredonia, N. Y. Copied on rectangular coördinate paper.

If the system of rectangular coördinates be used, the thermal-time value for any interval is given by an area; i. e., the area AOKB in Fig. 15. For purposes of comparison, it is necessary to resort to averages, so that when the term "daily mean temperature" is used we really mean the height of a rectangle which has the same area as that of the temperature curve. Since both surfaces have the

[1] In ecological studies (a term which embraces most of the growth and developmental studies of economic entomology, physiology of plants and animals, etc.) all temperature data are climatological. This is true whether the investigator observes these activities under natural conditions, or under controlled temperatures — in the latter instance he has only made an artificial climate. For this reason the adjective "climatological," when used in this treatise, will generally include this wider connotation. The expression "climatological worker" or "climatologist" will be used in reference to observers or investigators of meteorology.

same intercepts on the axis of abscissas (viz., the perpendiculars representing the interval of twenty-four hours, usually extending from midnight to midnight), obviously, the proper procedure would then be to find the area of the figure bounded by the temperature curve, the zero line, and the two perpendiculars (the surface AOKB in Fig. 15); and then construct a rectangle having the same area (for example, COKD). The accuracy of the daily mean would be limited only by the exactness of our determination of the area of the irregular figure, AOKB. Now, thermographs have not been in use for nearly as long a time as maximum and minimum thermometers, and, besides, owing to their expense, delicate nature, and the necessity of careful correction, are not in as general use as is warranted among biologists. To form temperature curves from thermometer observations during the day and night, certainly would be out of the question, and so the daily mean temperature, usually, is approximated by taking the average of the highest and lowest readings for each twenty-four hours, these being secured by accurate maximum and minimum thermometers. Furthermore, owing to the fact that these values seldom occur at midnight, the readings are made at a convenient hour of the day, which, for meteorologists, is generally at 8 P. M.

DISCREPANCY INVOLVED IN APPROXIMATING DAILY MEAN TEMPERATURE.

If the temperature curve were a straight line extending from midnight to midnight, the height of the equivalent rectangle would be given exactly by taking the average of the highest and lowest points. However, temperature curves for an entire day are practically never straight lines, so the averages of the maximum and minimum temperatures may, or may not (generally do not), give the exact area of the figure under the curves for each twenty-four hours. In Fig. 15, the rectangle, EOKF, represents the approximate area of the surface, AOKB, whereas, the true value is represented by the rectangle, COKD; therefore, the rectangle, ECDF, represents the discrepancy between the true and approximate areas of figure AOKB.

The justification for the use of maximum and minimum thermometers has been their cheapness, ease of manipulation, durability and accuracy. While they correctly indicate the highest and lowest temperatures in a given period, as just shown, an error is involved in assuming that the mean of the two readings will give, with precision, the daily mean temperature, and especially is this true if the readings are not made at midnight. However, it has been assumed by the meteorological users of these instruments that: (1) the average of the highest and lowest temperatures for the day will give a close approximation to the daily mean; (2) discrepancies

will generally be small, and will, in all probability, as often be too high as too low, so that, in the long run, positive and negative values will tend to neutralize each other, and, therefore, monthly or yearly means will be fairly close to the true value; (3) records taken at the same hour of the day will be comparable. The most common use of these instruments is by the coöperative observers of the U. S. Weather Bureau and, as will be shown subsequently, the discrepancies introduced in the monthly mean and annual mean temperatures are not of sufficient importance to necessitate a change to thermograph averages, since these observers are seldom less than thirty miles apart and, doubtless, the discrepancies are not as great as errors due to lack of uniformity of exposure. Within certain limits, the instruments must be located at the convenience of the observer, if records of any kind are to be secured and, in addition, more stress is placed upon monthly and yearly averages than on daily means.

Following the lead of the climatologists, there has been a tendency among biological workers to record only maximum and minimum temperatures, in their studies of climatological influences, and to make averages from such records even tho thermographs may be in use. Usually, daily thermal differences are to be studied in their relation to growth, reproduction or other activities of plants and animals, and, since the discrepancies between the true daily means and those calculated from maximum and minimum readings (especially if the hour of observation occurs rather early) may be large on certain days, serious errors may be introduced. It is the object of this study to indicate the extent of these variations.

CIRCUMSTANCES LEADING TO THE INVESTIGATION.

In December, 1915, the Station purchased and installed a Bristol Temperature Recorder at the Vineyard Laboratory, Fredonia. Previous to that time, all temperature records were secured from maximum and minimum thermometers, in the U. S. Weather Bureau pattern of shelter (Plate XXXIII, fig. 1), and the readings were made daily, at 5 P. M. The primary object of all our climatological data was in relation to entomological investigation, but the writer, incidentally, served the U. S. Weather Bureau in the capacity of cooperative observer.

A few words describing this instrument may be apropos. The recording portion of the apparatus (Plate XXXIV) consists of clockwork which moves a paper dial one revolution in seven days. The dial is divided by radiating lines into hours and days, while concentric circles, representing two degree intervals and extending from near the center to the circumference, give a range of from —20 to 110 degrees Fahrenheit. A pen, on a lever actuated by an expansion apparatus, has the possibility of movement over the entire range, the exact position at any moment being determined by the tempera-

ture at that moment affecting the portion of the instrument exterior to the building, and it thus records the temperature at any given time, while the movement of the dial traces the temperature curve continuously. Extending from the recording portion of the instrument to the exterior, is a very narrow copper tube about eight feet in length, ending in a large bulb of the same material enclosed in a shelter (Plate XXXIII, fig. 2). The bulb and tube being filled with alcohol, any difference in temperature — which causes a contraction or expansion of the bulb — increases or decreases the pressure on the alcohol, which, in turn, communicates motion to the recording lever. MacDougal[2] describes a similar bulb and tube which is connected with a drum type of thermograph, but the writer has never seen this device offered for sale by instrument makers.

The advantage of these bulb types of instruments over the ordinary thermograph[3] is that the recording part of the apparatus is conveniently located for observation, and is also protected from the elements which assists in keeping the charts clean and dry especially when changing them, while the bulb may be placed at any convenient distance from the building by varying the length of the copper tubing, thus insuring an accurate record of external temperatures.

After the thermograph was installed, observations with the maximum and minimum thermometers were continued in order that necessary corrections might be made. However, at the advice of the U. S. Weather Bureau, the report sent them was changed so as to extend from midnight to midnight, but the maximum and minimum temperatures were secured from the thermograph record (corrected) and no change in computing the means was made. Since we continued the 5 P. M. readings, comparisons were made of the two records for several months, and it was noted that discrepancies occurred. This aroused our curiosity, and a calculation of the daily mean temperatures, using the hourly temperatures (described below), revealed the fact that neither record, based on the means of the highest and lowest temperatures of each day, gave the same results as these computations. This investigation was carried out during the months of the entire year of 1916, and, as the results seem to be of importance, especially to biological investigators and perhaps to those studying climatology, they form the subject matter of this bulletin.[4]

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[2] MacDougal, D. T. The temperature of the soil. *J. N. Y. Bot. Garden*, 3:125–131. 1902.

[3] Thruout this bulletin the word *thermograph* will be used for any device giving a continuous record of temperature, and, if necessary, to distinguish the form of chart, the name *drum type* will refer to instruments having a revolving drum upon which the chart is attached, and *disc type* to those using a circular chart as shown in Plate XXXIV.

[4] Sanderson (*J. Econ. Ent.* 1:56–65. 1908) used thermograph records integrated by means of a planimeter, and has mentioned the fact that discrepancies occurred

DETERMINATION OF AREA OF TEMPERATURE CURVES AND UNITS USED.

As suggested by MacDougal,[5] the measurement of thermal values, for any day, is best accomplished by the use of a drum thermograph record, in which the area under the temperature curve is integrated by means of a planimeter, expressing the result as hourly-degree-units. He used the Centigrade scale, and introduced the term "hourly-Centigrade-units." Using the same method and notation with the system most common in this country, our results would be given as *hourly-Fahrenheit-degree units.* Division of the total by twenty-four gives the quotient as the average hourly-degrees for the day, which is nothing less than the mean daily temperature.

Owing to the radiating of the "vertical" lines of the dial type of chart or to the fact that a planimeter is not available for the drum type, recourse must be had to some other method of finding the *thermograph average.* This is accomplished most easily, if the curve is fairly uniform, by making a summation of the hourly temperatures, taking the precaution to note the average, and dividing the sum by twenty-four. This will be referred to as the *method of hourly temperatures.* However, when the changes are sudden, it is necessary to divide the hour intervals into several strips having equal bases, and from the areas of the several strips secure the average temperature for the hour. The method of hourly temperatures, while more laborious than that in which a planimeter is used, gives results practically as accurate if care be exercised. *In either method, the precision is comparable to the precision of the temperature curve.*

METHOD OF COMPARISON.

In making the comparisons, described subsequently, we assume that the mean daily temperature, calculated by the above methods, is the most exact obtainable; therefore all records for the same

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between the thermograph average and that based on the mean of the maximum and minimum temperatures. However he made no analysis of these differences.

Mr. C. A. Donnel, meteorologist, U. S. Weather Bureau, after reading the manuscript, called our attention to the following references to literature which considers some of the points emphasized in this bulletin:

Bigelow, F. H. Report on the Temperatures and Vapor Tensions of the United States. Bulletin S, U. S. Weather Bureau. 1909.

Donnel, C. A. The Effect of the Time of Observation on Mean Temperatures. Monthly Weather Review 40:708. 1912.

While the writings discuss some of the phases covered by the present treatise, no attempt has been made to analyse the daily data for an entire year at any one station, as well as changes from day to day. In whatever way the author's investigation overlaps those of other investigators, the evidence is corroborative. Furthermore the primary object of this study was to call attention to the use of more accurate thermal data in biological investigation, a feature which the writer has not seen sufficiently emphasized in the literature.

[5] Loc. cit., pp. 125–131.

date have been referred to this value as a "base" or "datum." All differences from this fundamental value are designated *discrepancies*, and the direction of the difference is indicated by the usual algebraic sign: a value higher than the base is marked plus (+); a value lower, minus (—).

The first step was to compute the thermograph average for each day of 1916. Then the daily mean temperatures were calculated for each day, using the maximum and minimum temperatures for each twenty-four hours, ending respectively at 5 P. M., 8 P. M., and midnight,[6] which are called the 5 P. M. Average, 8 P. M. Average and Midnight or 12 P. M. Average. Having these values, the discrepancies between the thermograph average and the several other averages were found. The corrected thermograph records were used to secure all data, so that the different values obtained are due to the method of computing and not to instrumental errors.

In analyzing the data, we have used three periods: the year, the successive months and the days of the month. The deductions drawn from the analysis of the data of each period have a practical bearing on the use that is to be made of the records. and, for that reason, are treated separately.

ANALYSIS OF DAILY DISCREPANCIES DURING A YEAR.

FREQUENCY DISTRIBUTIONS OF DISCREPANCIES.

The most satisfactory method of analyzing any mass of data is by statistical methods. First, to bring out the differences of the mean, standard deviation (spread of the curve) and range of each series, the discrepancies have been grouped by two degree intervals into frequency distributions and the polygons constructed, superimposing the three (Fig. 16). It is to be noted that the three polygons have the same area.

From the distributions, the following deductions and comparisons may be made: (1) Small discrepancies occur more frequently than large ones; (2) the time of observation determines the probability of large deviations, 5 P. M. producing many more large values than observations taken later in the day; (3) positive discrepancies are of more frequent occurrence than negative ones — so these

[6] It will be noted that the actual periods of time covered for the same date were not identical in every case. For example, take Feb. 24, the period of time used to compute the thermograph and midnight averages were the same; the period for the 8 P. M. average extended from 8 P. M. of the 23d to 8 P. M., Feb. 24; and that for the 5 P. M. average from 5 P. M., Feb. 23, to 5. P M., Feb. 24. In the midnight average there is introduced the discrepancy due to calculation, whereas in the other two averages there are discrepancies of calculation, and, in addition, errors are included owing to the fact that the durations of time, while equal, do not extend over the same interval as the thermograph average.

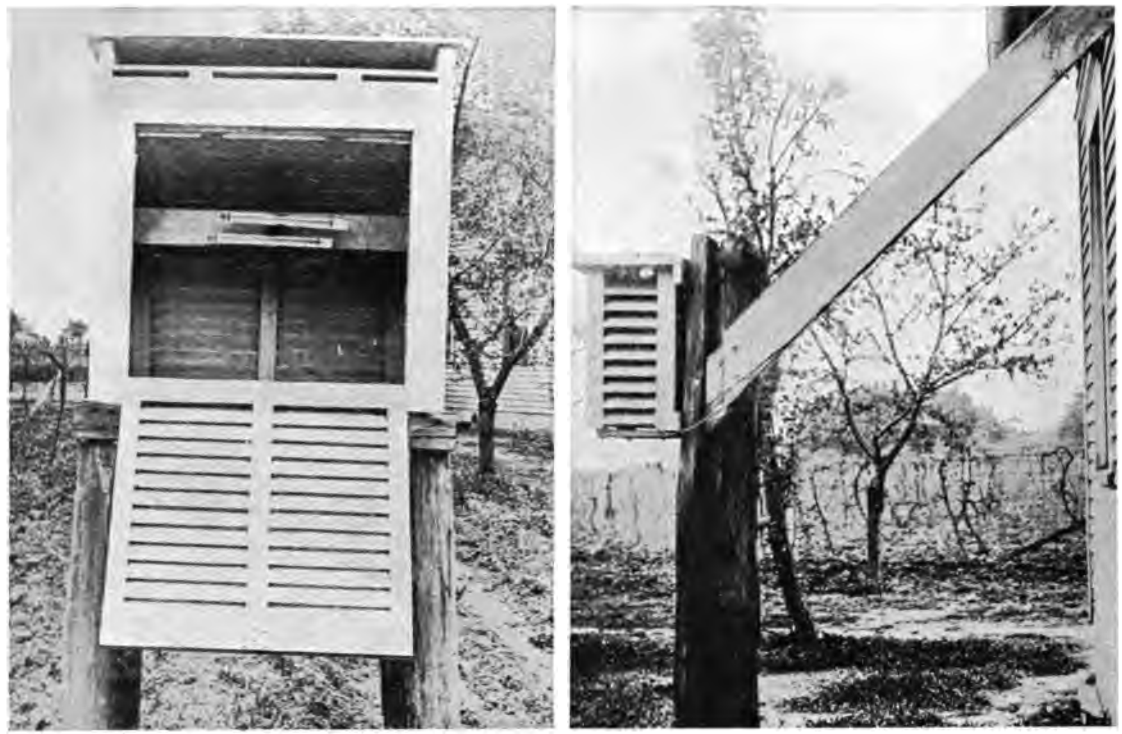

Plate XXXIII.— Shelters for Meteorological Instruments at Fredonia, N. Y.

1. U. S. Weather Bureau Shelter with Maximum and Minimum Thermometers.
2. Shelter Containing Bulb of Thermograph with Protected Connecting Tube.

PLATE XXXIV.— DIAL TYPE OF THERMOGRAPH AT VINEYARD LABORATORY, FREDONIA, N. Y.

discrepancies do not neutralize each other,— thus causing the means to be positive, this tendency being the greater the earlier the observations; (4) the empirical mode of all the observations has the same value; (5) the greatest differences are found in the height of the curve, the location of the mean, the "scatter" of the observations and the range; and (6) the greatest differences occur in the positive portions of the curves. It will readily be seen that the computations based on the midnight average give the closest approximation to the thermograph average, followed in turn by the 8 P. M. and 5 P. M. averages, and that no method based on maximum and

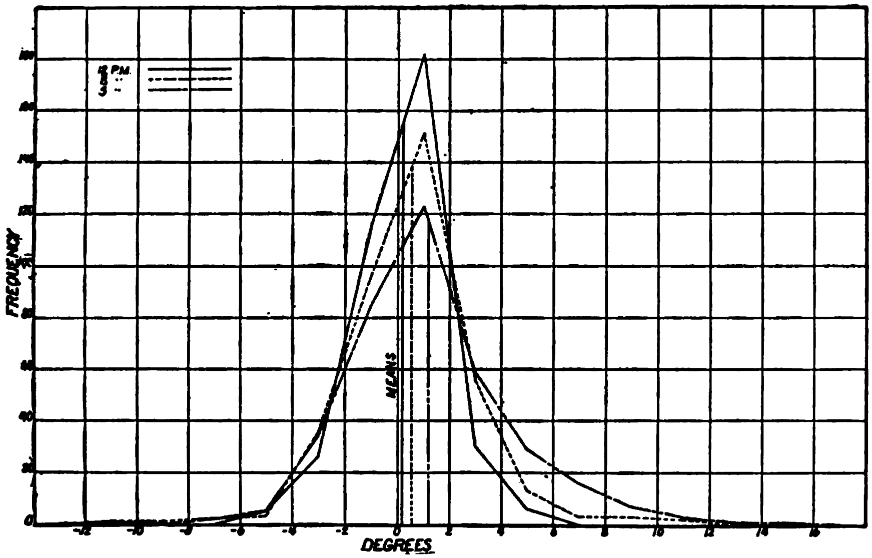

FIG. 16.— Comparison of frequency polygons of discrepancies in daily mean temperatures computed from the three records by averaging the highest and lowest values.

minimum temperatures is as exact as that based on hourly temperatures.

In order to calculate the statistical constants, as well as to show the distributions more accurately, it is necessary to use different units of grouping in the several series. The discrepancies have been calculated to one-tenth of a degree F., but, since the range in each record is rather large, it was necessary to group the values. In doing this, the aim was to make the number of groups not less than twelve nor more than eighteen (for reasons that are discussed in elementary works on statistics). Convenient groupings were found to be: for the 5 P. M. discrepancies, 2.0 degrees; for the 8 P. M.

values, 1.5 degrees; and for those of midnight, 0.8 degrees. Incidentally, this gave fourteen groups for each distribution. The several distributions are given in Table I, and the frequency polygons are shown in Figures 17 to 19. It will be noted that all the distributions tend to be symmetrical, and that the empirical mode of each falls in the positive portion of the curve.

TABLE I.— FREQUENCY DISTRIBUTIONS OF DAILY DISCREPANCIES DURING 1916. FREDONIA, N. Y.

MIDNIGHT AVERAGE.		8 P. M. AVERAGE.		5 P. M. AVERAGE.	
Mid values (Deg. Fahr.)	Frequency.	Mid values (Deg. Fahr.)	Frequency.	Mid values (Deg. Fahr.)	Frequency.
—5.2	1	—9.75	1	—11	1
—4.4	4	—8.25	1	— 9	0
—3.6	5	—6.75	2	— 7	2
—2.8	10	—5.25	1	— 5	5
—2.0	24	—3.75	11	— 3	34
—1.2	45	—2.25	37	— 1	85
—0.4	59	—0.75	85	1	123
0.4	91	0.75	119	3	59
1.2	66	2.25	73	5	29
2.0	40	3.75	19	7	16
2.8	12	5.25	9	9	7
3.6	3	6.75	3	11	3
4.4	4	8.25	2	13	1
5.2	2	9.75	3	15	1
Total......	366		366		366

In the statistical analysis, the methods of Pearson have been used. These methods have been summarized by C. B. Davenport,[7] Elderton,[8] E. Davenport,[9] and Pearl and Surface,[10] while the tables used were those published by Pearson.[11] These have been used in our study, and the calculations have been carried out chiefly by means of logarithms. The work has been checked by duplicate calculation, and it is hoped that serious errors have been avoided. In Table II are given the analytical constants for the distributions of Table I.

[7] Davenport, C. B., Statistical Methods with Special Reference to Biological Variation. 2d Ed. New York. 1904.

[8] Elderton, W. P., Frequency Curves and Correlation. London. 1907.

[9] Davenport, E., Principles of Breeding. Boston. 1907.

[10] Pearl, R., and Surface, F. M., A Biometrical Study of Egg Production in the Domestic Fowl. Bul. 110, Bur. Animal Ind., U. S. Dept. Agr., Pt. I, 1909; Pt. II, 1911; and Pt. III, 1914.

[11] Pearson, Karl., Tables for Statisticians and Biometricians. Cambridge. 1914.

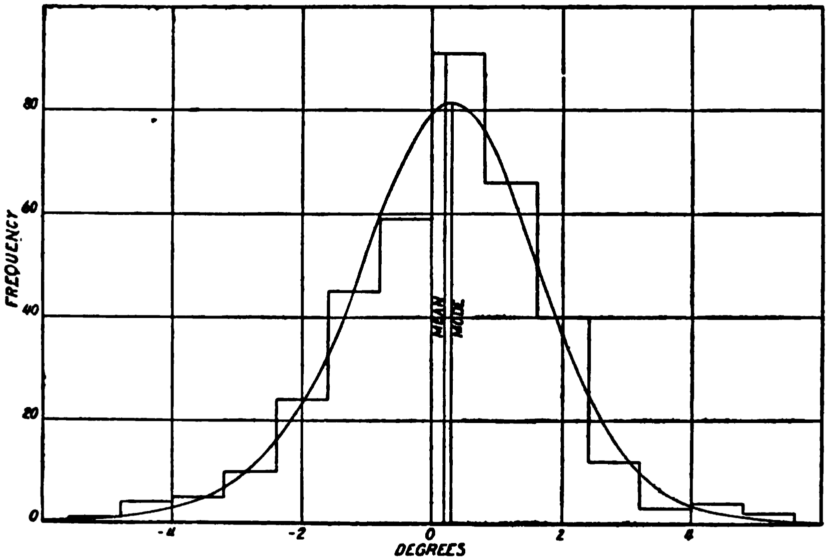

FIG. 17.— Histogram and fitted curve of variation in daily discrepancy, 12 P. M. record.

TABLE II.— ANALYTICAL CONSTANTS OF VARIATION OF DISCREPANCIES IN DAILY MEAN TEMPERATURES.

Constant.	12 P. M.	8 P. M.	5 P. M.
Mean	.2011	.5820	1.2131
Standard deviation	1.5835	2.3311	3.0916
μ_2	3.9177	2.4150	2.4326
μ_3	—1.6587	.8602	2.6617
μ_4	64.8123	38.5897	30.3825
β_1	.0458	.0525	.4921
β_2	4.2228	6.6164	5.1342
$\sqrt{\beta_1}$	.2139	.2292	.7015
β_2-3	1.2228	3.6164	2.1342
κ_2	.0154	.0065	.1530
Skewness	— .0653	+ .0464	+ .2080
Modal divergence	.1033	.1082	.6488
Mode	.3044	.4738	.5643
Type of curve	IV	IV	IV
+ end of curve	+ ∞	+ ∞	+ ∞
— end of curve	— ∞	— ∞	— ∞
Origin	.7311	.2189	—1.9748

The moments are given in terms of the units of grouping, which have been stated above, while the mean, mode, origin and standard deviation of each distribution are in degrees.

It will be noted that in all the distributions κ_2 is positive, greater than zero and less than 1. This would indicate that Pearson's Type IV curve is demanded and, in fact, has been used to fit all distributions. However, the value of the criterion (κ_2) in both the midnight and the 8 P. M. series is so small, in comparison with its probable error, that one might be led to believe that some type of symmetrical curve would graduate the frequencies. Especially considering that the skewness in both instances is insignificant, and the fact that β_1 in each case is small, we first fitted the distributions by means of Pearson's Type VII curve, but, in each distribution, the fit was so poor that we discarded this type for Type IV, which gave better graduations.

All the distributions are unusual, since the point $\beta_1 \beta_2$ of two of these falls very near the limiting parabola $\beta_6 = \infty$ in Pearson's diagram,[12] while for the 8 P. M. discrepancies this point falls beyond this limit, and, therefore, the curve is heterotypic. This feature is, no doubt, the disturbing element which caused Type VII curves to fit so poorly.

The equations of the theoretical curves are:

12 P. M.:

$$y = \frac{77.4389}{\left\{1 + \left(\dfrac{x}{4.2329}\right)^2\right\}^{5.1291}} \cdot \frac{1}{e^{1.0339 \tan^{-1} x/4.2329}}$$

8 P. M.:

$$y = \frac{110.9234}{\left\{1 + \left(\dfrac{x}{4.4805}\right)^2\right\}^{3.3592}} \cdot e^{.3324 \tan^{-1} x/4.4805}$$

5 P. M.:

$$y = \frac{61.1028}{\left\{1 + \left(\dfrac{x}{7.5012}\right)^2\right\}^{4.9135}} \cdot e^{3.3263 \tan^{-1} x/7.5012}$$

The histograms and their fitted curves are shown in Figures 17–19. The several graduations are fairly close to the observed frequencies, and the fit, in each instance, is as good as could be expected.

The area of the fitted curves was calculated by Simpson's quadrature formula

$$\int_0^1 y_x dx = \frac{1}{6}\,(y_0 + 4y_{\frac{1}{2}} + y_1).$$

[12] Pearson, Karl, Tables for Statisticians and Biometricians, p. 66.

From Figures 17–19 and Table II, the following features may be emphasized:

All the curves are more peaked than normal; the kurtosis ($\eta = \beta_2 - 3$) being significantly positive for all since the probable error of β_2 in each distribution[13] is: midnight, $\pm .1716$; 8 P. M., $\pm .1722$, and 5 P. M., $\pm .1625$. Thus all these curves are leptokurtic.

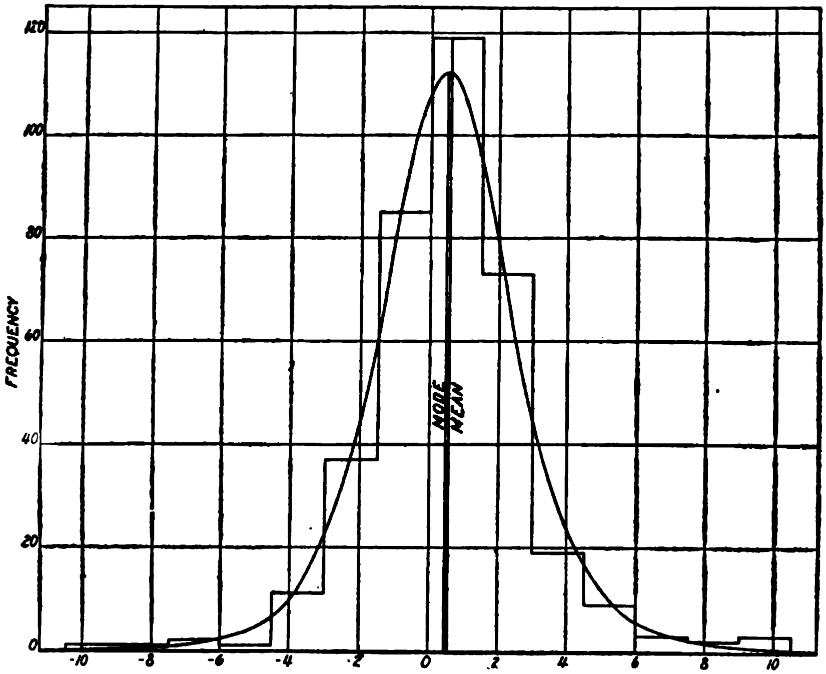

FIG. 18.— Histogram and fitted curve of variation in daily discrepancy, 8 P. M. record.

The skewness of the midnight distribution is negative, but positive for the other two. However, the value is small and practically insignificant in the 8 o'clock distribution. In the 5 P. M. curve the skewness is significant.

The modal divergence — the distance between the mean and the mode — is significant in two of the distributions but not in that of the midnight discrepancies.

---

[13] The following formulas were used to determine the probable errors of the skewness and β_2: P. E. Sk $= \pm .6745 \sqrt{\frac{3}{2N}} \frac{1}{\sqrt{1 + 3\,(\text{Sk})^2}}$ and P. E. $\beta_2 = \pm 4$ P. E. Sk. A value is not considered significant unless it is at least three times its probable error.

RELATIVE ACCURACY OF THE SEVERAL RECORDS.

There are a number of measures of accuracy that may be applied to the distributions. From a practical viewpoint the most important are: the effect of the discrepancies on the annual mean temperature; and, the expected number of days showing extreme deviation from the thermograph average. Other measures are to be found in comparisons of the standard deviation, probable discrepancy for a single day (page 419), and the range of the distributions. In making comparisons, the distribution showing the smallest values for each of these measures is the most accurate.

Effect of the discrepancies on the annual mean temperature.— The annual mean temperature is of especial interest to three groups: climatologists, phytogeographers and ecologists who are interested in the distribution of animals. It is true that mean temperatures tell only a part of the story of plant and animal distribution, but considerable use is made of them. The problem before us is to determine the relative discrepancies that occur in the annual mean temperatures as calculated from daily means taken at different hours. The data are set forth in Table III.

TABLE III.— AVERAGE DISCREPANCY IN THE MEAN ANNUAL TEMPERATURE COMPUTED FROM DAILY MAXIMUM AND MINIMUM RECORDS.

(Degrees Fahr.)

Record	Mean discrepancy	Comparisons		
		Records compared	Difference	Difference divided by probable error
Thermograph....	0	Thermograph & 12 P. M.	0.2011±.0558	3.6
Midnight........	0.2011±.0558	Thermograph & 8 P. M.	0.5820±.0822	7.1
Eight P. M......	0.5820±.0822	Thermograph & 5 P. M.	1.2131±.1090	11.1
Five P. M.......	1.2131±.1090	Midnight & 8 P. M......	0.3809±.0994	3.8
		Midnight & 5 P. M......	1.0120±.1225	8.3
		Eight P. M. & 5 P. M...	0.6311±.1365	4.6

The following conclusions have been drawn: (1) All differences are significant, statistically considered; (2) no method of calculating daily mean temperatures gives as accurate annual mean temperature values as that based on hourly temperatures; (3) the greater the period previous to midnight that the record is taken, the larger is the average discrepancy; taking the midnight record as unity, they stand roughly in the ratio of 1: 3: 7; (4) from a practical viewpoint, averages of daily maximum and minimum temperatures when made not earlier than 8 P. M., affect the annual mean temperature so slightly that the differences are negligible; (5) the 5 P. M. averages give differences that may be important, so records should be taken at a later hour.

Standard deviations compared.— The best measure of the relative accuracy of the several distributions is furnished by a comparison of the standard deviations of the same. This has been done in Table IV.

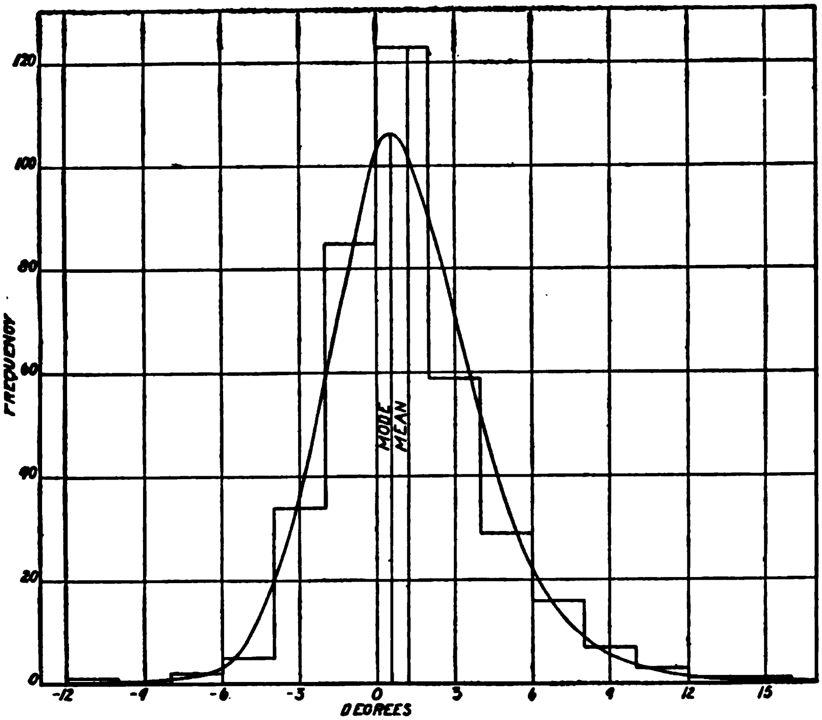

FIG. 19.— Histogram and fitted curve of variation in daily discrepancy, 5 P. M. record.

TABLE IV.— COMPARISONS OF THE STANDARD DEVIATIONS OF THE SEVERAL DISTRIBUTIONS.

(Degrees Fahr.)

Record	Standard deviation	Comparisons		
		Records compared	Difference	Difference divided by probable error
Thermograph....	0	Thermograph & 12 P. M.	1.5835±.0395	40.1
Midnight........	1.5835±.0395	Thermograph & 8 P. M.	2.3311±.0581	40.1
Eight P. M......	2.3311±.0581	Thermograph & 5 P. M.	3.0916±.0770	40.2
Five P. M.......	3.0916±.0770	Midnight & 8 P. M......	0.7476±.0703	10.6
		Midnight & 5 P. M......	1.5081±.0865	17.4
		Eight P. M. & 5 P. M...	0.7605±.0965	7.9

If the value of unity be assigned to the standard deviation of the midnight record, the three values stand in the proportion of 1:1.5:2. In order to bring out differences, comparisons are included. All differences are significant and, of the records in which maximum and minimum temperatures have been used, that of midnight is the most accurate, followed by the eight o'clock record, while the five o'clock averages are the least precise.

Range.— It is generally admitted by statistical workers that the range is the least desirable of the measures of variation. However, a comparison of the range in the several distributions will bring to light the tendency of certain records to give extreme variants (Table V).

TABLE V. VARIATION IN RANGE OF DISCREPANCIES IN THE SEVERAL DISTRIBUTIONS.
(Degrees Fahr.).

Record.	Positive.	Negative.	Total.
Midnight	5.3	5.0	10.3
8 P. M.	10.1	10.0	20.1
5 P. M.	14.6	10.5	25.1

These figures still further corroborate the deductions given above.

CONCLUSIONS FROM STUDY OF ANNUAL DATA.

In view of the facts that have been discussed above, it appears that, *so far as the annual mean temperature is concerned*, observers using maximum and minimum thermometers can secure sufficiently accurate results by the use of these instruments, providing the records are not taken too early. Considering the convenience to the observer and the precision of the data, eight P. M., the hour generally used by coöperative observers of the U. S. Weather Bureau, is a desirable time to make the records. For comparative purposes, it would be best to have all observations, except those based on thermograph records, made at this hour.

ANALYSIS OF DAILY DISCREPANCIES BY MONTHS.

The daily discrepancies of each average are shown by months in Figs. 20 to 31, inclusive. A study of these graphs reveals certain facts which may be enumerated.

The most extreme discrepancies occurred during the months from January to March inclusive and December, while the smallest divergences are found during the period from June to September inclusive. April, May, October and November show intermediate extremes.

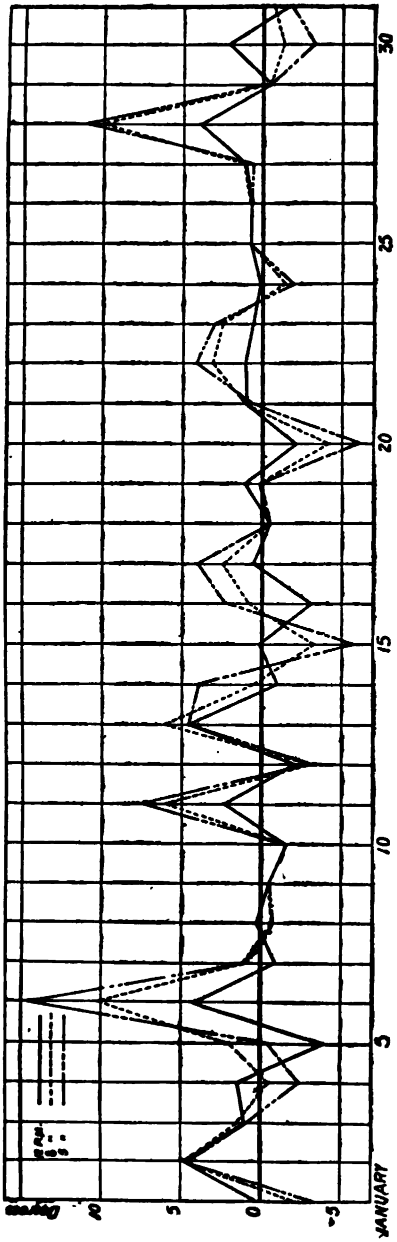

FIG. 20.—Diagram showing fluctuation in daily discrepancies for the three records, January, 1916. Fredonia, N. Y.

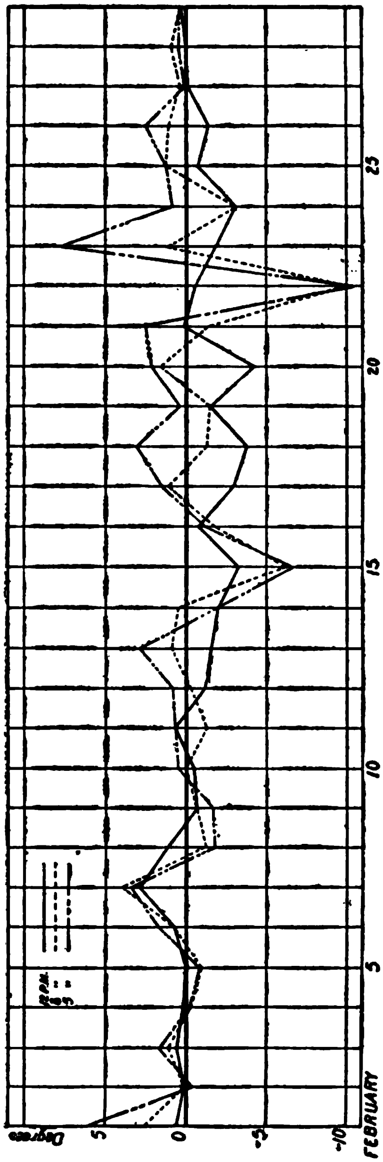

FIG. 21.— Diagram showing fluctuation in daily discrepancies for the three records, February, 1916. Fredonia, N. Y.

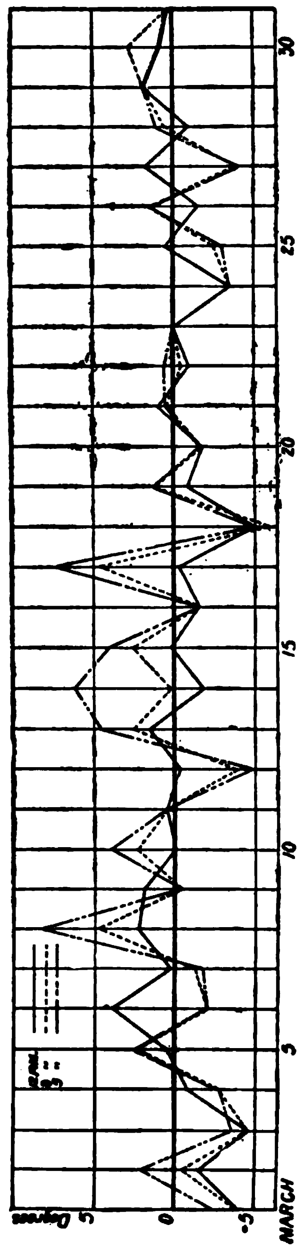

FIG. 22.— Diagram showing fluctuation in daily discrepancies for the three records, March, 1916. Fredonia, N. Y.

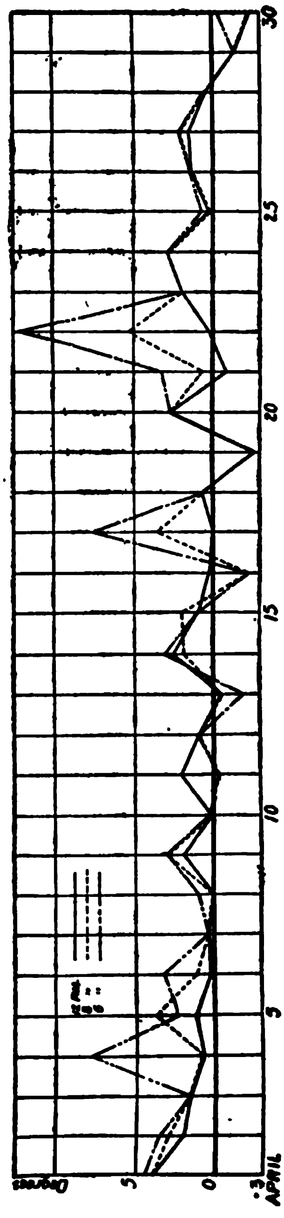

FIG. 23.— Diagram showing fluctuation in daily discrepancies for the three records, April, 1916. Fredonia, N. Y.

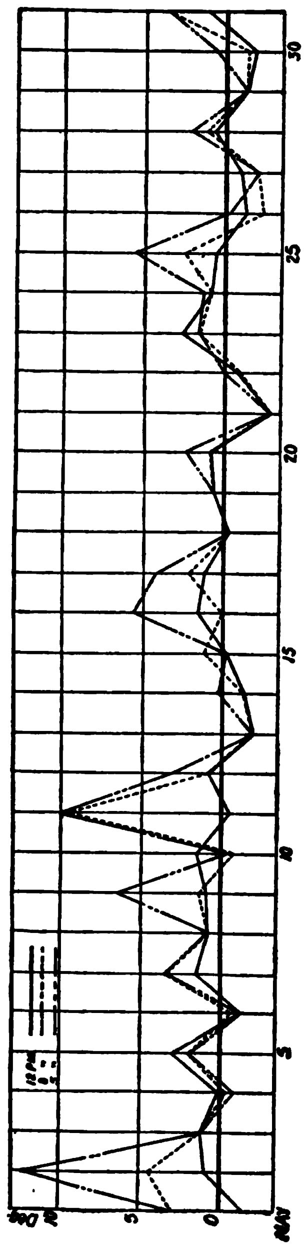

FIG. 24.— Diagram showing fluctuation in daily discrepancies for the three records, May, 1916. Fredonia, N. Y.

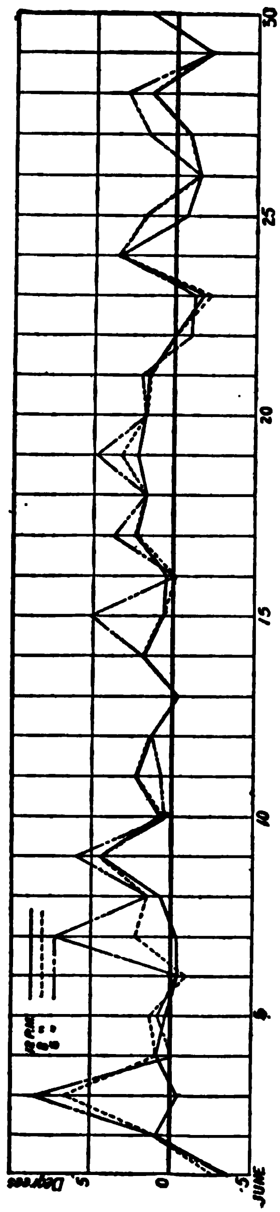

FIG. 25.— Diagram showing fluctuation in daily discrepancies for the three records, June, 1916. Fredonia, N. Y.

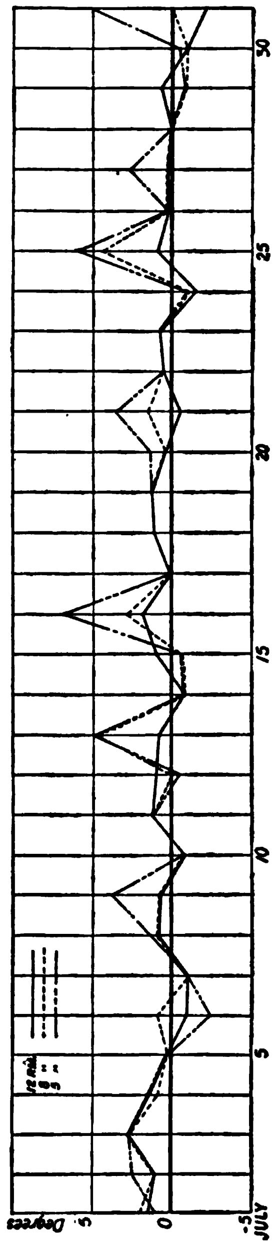

FIG. 26.— Diagram showing fluctuation in daily discrepancies for the three records, July, 1916. Fredonia, N. Y.

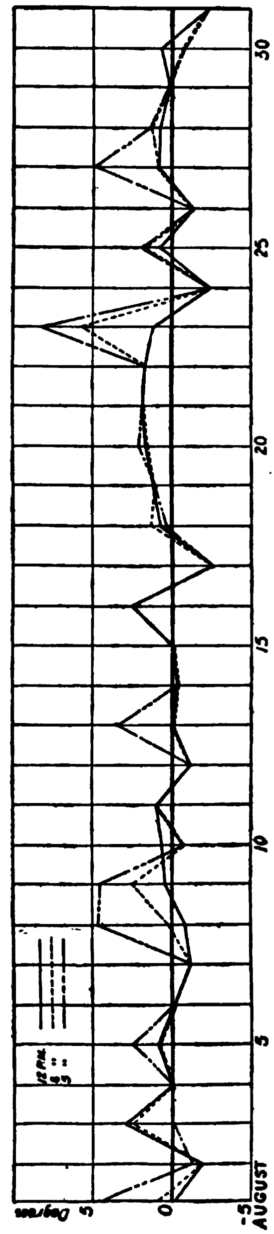

FIG. 27.— Diagram showing fluctuation in daily discrepancies for the three records, August, 1916. Fredonia, N. Y.

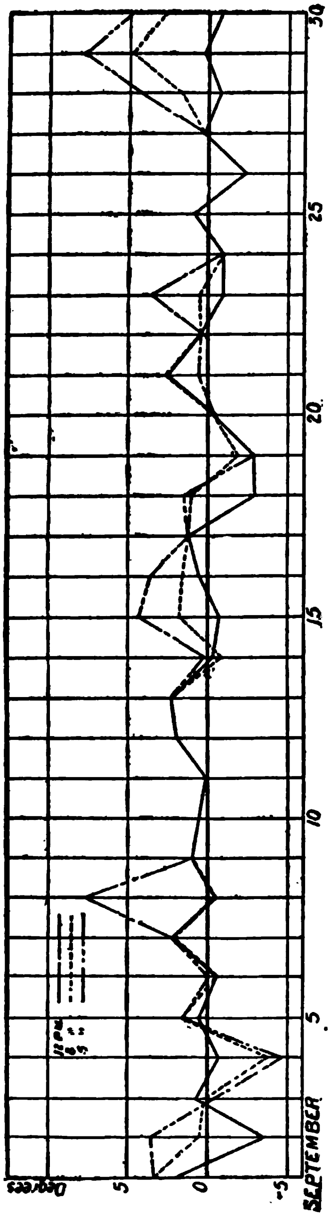

FIG. 28.— Diagram showing fluctuation in daily discrepancies for the three records, September, 1916. Fredonia, N. Y.

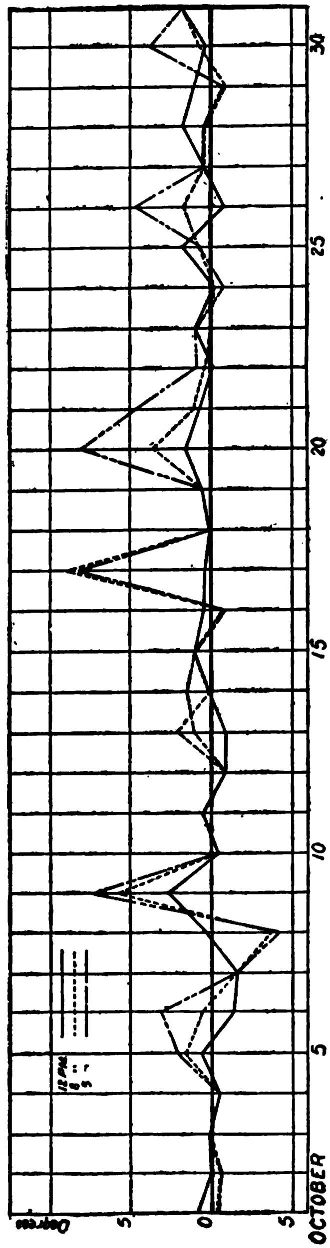

FIG. 29.— Diagram showing fluctuation in daily discrepancies for the three records, October, 1916. Fredonia, N. Y.

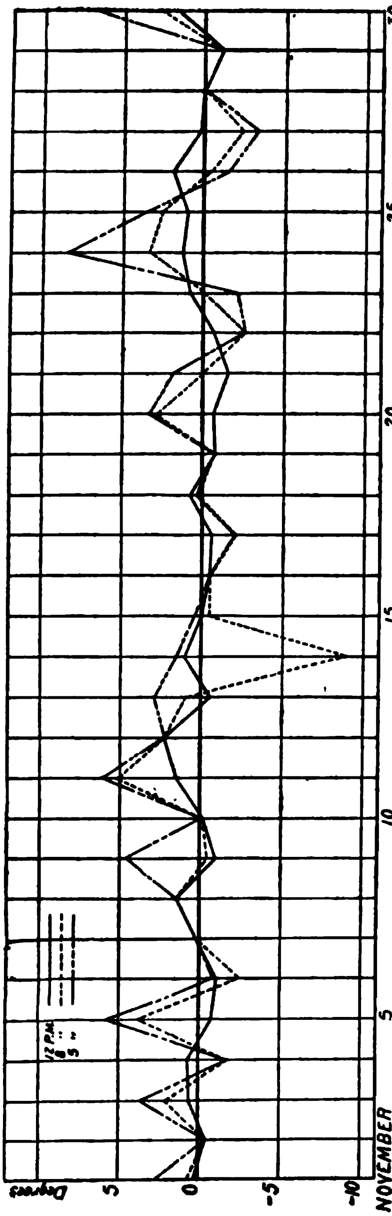

FIG. 30.— Diagram showing fluctuation in daily discrepancies for the three records, November, 1916. Fredonia, N. Y.

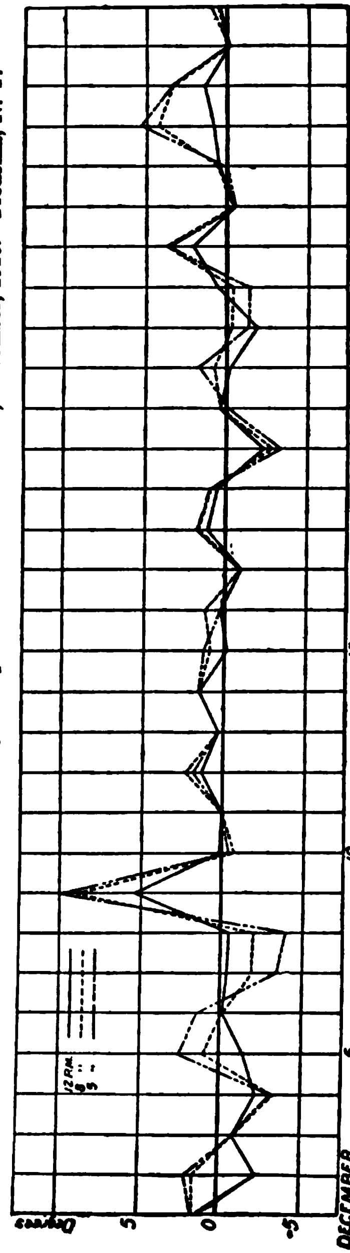

FIG. 31.— Diagram showing fluctuation in daily discrepancies for the three records, December, 1916. Fredonia, N. Y.

Usually, the majority of divergences are positive, but February has an excess of negative values, and March appears to be nearly equally divided in the curve for five o'clock, while the other differences are mostly negative, and July shows the smallest deviations.

In a study of the daily variations from the thermograph average during the successive months, the most important relation to be considered is the effect of the discrepancies upon the monthly mean temperature, but, incidentally, the range and average value (disregarding signs) of these deviations are worthy of study.

AVERAGE MONTHLY DISCREPANCY.

From a practical standpoint, the main problem to be solved is the effect of the discrepancies in any record on the monthly mean temperatures. To determine this, an average of the discrepancies has been computed for each month, paying due regard to signs.

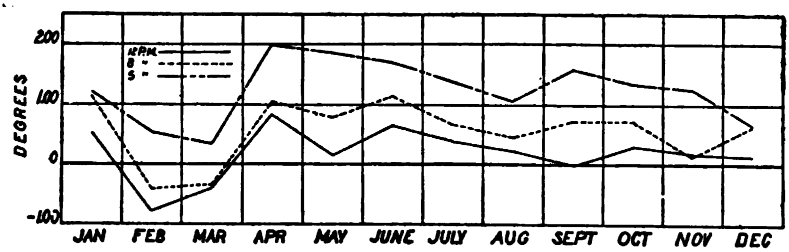

FIG. 32.— Graph showing the variation in average monthly discrepancies during 1916. Fredonia, N. Y.

Table VI gives the data, while Fig. 32 shows the same graphically.

These data may be summarized as follows:

The 5 P. M. record gives positive average discrepancies for every month, which indicates that the monthly mean temperatures were always too high. Generally, the values fluctuated from one to two degrees, altho, for February and March they were only about one-half of a degree above the thermograph average.

The midnight discrepancies gave positive average values for nine months, and during ten of the months showed the least deviation from the "base," the values usually ranging from one-half of a degree to near zero. This record also gave the lowest values of the three in a vertical scale, and, curiously, showed greater deviations from the thermograph average during February and March than did those of five and eight o'clock, because the signs happened to be negative.

The eight o'clock average produced discrepancies greater than one degree for only three of the months, and was uniformly higher than the midnight average except in November, when it was slightly

TABLE VI.— AVERAGE MONTHLY DISCREPANCIES FROM THERMOGRAPH RECORD.

(Degrees Fahr.).

Record	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Midnight.....	.52	—.78	—.40	.82	.15	.63	.39	.22	—.01	.31	.19	.14
Eight P. M...	1.13	—.40	—.36	1.04	.78	1.13	.68	.45	.72	.72	.14	.63
Five P. M....	1.21	.53	.34	1.99	1.86	1.68	1.39	1.05	1.57	1.33	1.26	.67

TABLE VII.— MONTHLY MEAN DISCREPANCIES FROM THERMOGRAPH RECORD.

(Degrees Fahr.).

Record	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Midnight.....	1.66	1.29	1.50	1.32	1.22	1.43	.90	1.14	1.18	.76	.88	1.05
Eight P. M...	2.45	1.63	2.20	1.79	1.91	1.79	1.10	1.40	1.51	1.34	1.74	1.65
Five P. M....	3.22	2.30	2.71	2.58	2.61	2.38	1.91	2.00	2.26	2.02	2.43	1.94

TABLE VIII.— RANGE IN DISCREPANCIES DURING EACH MONTH, 1916.

(Degrees Fahr.).

Record	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Midnight....	—4.0 to 4.8	— 4.3 to 2.9	—5.0 to 3.9	—2.6 to 3.9	—2.9 to 3.0	—3.7 to 4.4	—2.0 to 2.7	—2.6 to 2.9	—3.5 to 2.7	—1.6 to 2.7	—1.6 to 2.2	—2.4 to 5.8
Eight P. M...	—4.1 to 10.1	—10.0 to 3.9	—6.0 to 4.7	—2.6 to 5.2	—2.9 to 9.5	—2.7 to 6.6	—1.0 to 4.8	—2.6 to 5.7	—3.7 to 4.8	—3.7 to 8.9	—8.9 to 5.1	—3.3 to 8.8
Five P. M....	—6.1 to 14.6	—10.5 to 7.7	—5.0 to 8.2	—2.6 to 11.7	—2.9 to 12.4	—3.7 to 8.6	—2.4 to 6.9	—2.6 to 8.2	—4.7 to 7.8	—4.2 to 8.1	—3.3 to 8.4	—4.0 to 9.8

lower than the latter. In general, the former produced values but slightly higher than those given by the midnight average.

MONTHLY MEAN DISCREPANCY.

The use of the *mean deviation* is common. This is computed by taking the sum of the deviations from the mean, disregarding signs, and dividing by the number of observations. We propose to use a somewhat analogous average, which we have termed the *monthly mean discrepancy*. It is calculated by taking the sum of the daily discrepancies (disregarding signs) for each day of the month and dividing by the number of days. Of two records, the one showing the higher mean is the least reliable. This has been done for the three records for the successive months of 1916 (Table VII). The results are shown graphically in Fig. 33. The quotients were found to vary markedly during the several months, the highest values being found, generally, during the first four months. There is also

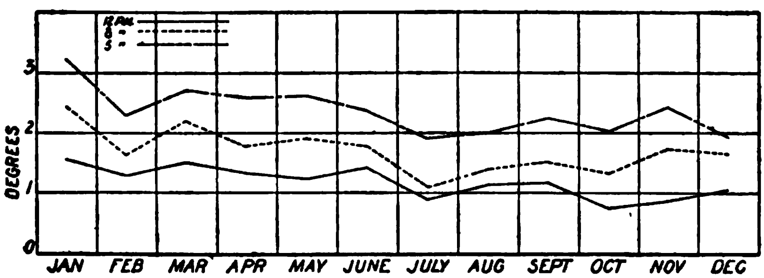

FIG. 33.— Graph showing the variation in mean monthly discrepancies during 1916. Fredonia, N. Y.

an indication of periodic variation. The parallelism exhibited by the several curves is conspicuous, showing that the relative accuracy remains nearly constant during the different months. As was found in the annual summary, earlier records have proved least trustworthy, and none have equalled the thermograph averages in precision.

RANGE.

A comparison of the range of deviations (Table VIII) from the datum, substantiates the conclusions deduced in the previous paragraph. In the midnight record the range of discrepancy is generally small, and the upper and lower values tend to be rather uniform during the different months. The greatest range in discrepancy occurred each month in the five o'clock averages, and the negative values, as a whole, were smaller and more uniform than the positive values. The negative discrepancies of the eight and five o'clock records were similar, but the positive values were decidedly higher in the latter.

SUMMARY OF STUDY OF MONTHLY DATA.

As will be observed from Tables VI to VIII inclusive, no method of computing mean monthly temperatures from maximum and minimum readings is as accurate as that calculated from hourly temperatures. For meteorological purposes, records taken at eight o'clock in the evening appear to be the most desirable if maximum and minimum thermometers are in use, since the errors of computation introduced are not excessive, and the hour is convenient for the observer. When the variation of the exposure of the instruments is considered, it is doubtful whether any important gain in the accuracy in the mean temperature for a month would be secured by furnishing coöperative observers with thermographs. However, biological workers should realize the extent of error to be expected, and if accurate results are desired, they should use thermographs, and secure the averages by means of a planimeter or the summation of hourly temperatures.

COMPARISON OF DAILY TEMPERATURE AVERAGES COMPUTED FROM THE SEVERAL RECORDS.

While climatologists are especially interested in the yearly and monthly means, the investigator, who is studying the effect of thermal influence on living organisms out of doors, is keenly concerned with the relation of the amount of heat and temperature changes to be found from day to day. For his use, it is desired that all thermal data be accurate, otherwise relationships of heat to the activities of plants and animals may be masked by the errors of the data. A glance at Figs. 20 to 31 will indicate at once the unreliability of any of these approximate methods of indicating the mean temperature for each twenty-four hours, if exact results are desired. The curves exhibit marked irregularity during any month, showing that there is no means of predicting the amount of divergence to be expected from day to day. There were very few times that any of the averages were the same as the thermograph average, i.e., when the curve touched the zero line.[14]

NUMBER OF DAYS SHOWING EXCESSIVE DEVIATIONS FROM THE THERMOGRAPH AVERAGE.

A comparison can be made by determining the number of days that gave discrepancies greater than some value which we may decide upon as being excessive. This value would vary with the nature of the use that is to be made of the records, but for biological purposes we would believe that a discrepancy of five or more degrees either above or below the thermograph average would be

[14] The crossing of the zero line in passing from positive to negative values, or vice versa, should not be confused with the days when the discrepancies were zero.

considered as excessive, and so this value has been used. Two methods of determining the number of days are available: counting the actual number observed in each record, and calculating the area of the portion of the curves (Figs. 17–19) lying beyond the ordinates erected at the points +5 and —5. The latter value we will call the *expected* number of days. The data are exhibited in Table IX, which needs no explanation. It may be interesting, however, to add that on nearly one-third of the days (110, to be exact) the three records gave identical discrepancies; i.e., the three curves coincided on these dates.

TABLE IX.— NUMBER OF DAYS DURING 1916 HAVING DISCREPANCIES OF FIVE DEGREES OR OVER. FREDONIA, N. Y.

Record	Discrepancies.				
	Kind.	Positive.	Negative.	Total.	Difference observed and expected.
Midnight	Observed	1	1	2	0
	Expected	1	1	2	
8 P. M.	Observed	12	4	16	0
	Expected	12	4	16	
5 P. M.	Observed	34	5	39	5
	Expected	39	5	44	

PROBABLE DISCREPANCY FOR A SINGLE DAY.

In order to further illustrate the accuracy of the several records, an answer will be sought to the question: on the average, how great differences may we expect in each day's mean when computed from readings taken at the different hours? This is analogous to determining the probable error[15] of a single observation, and is calculated in the same manner: viz., by multiplying the standard deviation by ±.6745. The results are given in Table X.

TABLE X.— PROBABLE DISCREPANCY FOR A SINGLE DAY.
(Degrees Fahr.)

Thermograph average	0
Midnight average	±1.0684
Eight P. M. average	±1.5723
Five P. M. average	±2.0853

[15] A discrepancy, in fact, is an error, but the term *error*, as used in statistics, has a different meaning, and for this reason, the use of the word has been carefully avoided, and the term *discrepancy* has been used in this study.

It is to be stressed that these differences may be either too high or too low and bear out the deductions made above regarding the three series.

CONCLUSIONS FROM STUDY OF DAILY DATA.

The upshot of all this analysis is, that the earlier the records are taken the less confidence can one place in the averages and that, for daily comparisons, maximum and minimum readings are of very doubtful value for investigators of either climatology or biology.

APPLICATION OF THIS STUDY TO BIOLOGICAL INVESTIGATION.

Attempts to measure the effects of thermal influence on the growth or reproductive activity of plants and animals have led to various uses of the daily mean temperature, a common one being to compare the actual measured activity with the mean temperature of the day; for example, the number of eggs laid by an insect from day to day is compared with the mean temperatures for the same days. It is obvious from Figs. 20 to 31 that discrepancies in the temperature data are apt to mask true relationships.

Another point of attack upon the same problem is to compare differences in temperature from day to day with differences in activity of the subjects being studied. Still another use of daily temperature data is to determine the number of daily-degree heat units necessary to cause a certain phenomenon to occur, such as the emergence of an insect from hibernation or the blossoming or leafing of a certain variety of plant. The extent to which discrepancies in daily temperature affect such calculations of thermal influence will now be discussed.

COMPARISON OF DAILY CHANGES AS GIVEN BY THE SEVERAL RECORDS.

Since the records of the entire year would unnecessarily consume space, it was decided to give comparisons during two important months (biologically considered) in the State of New York: May and June. The differences in daily mean temperature between the successive days of the months have been found for the four records and tabulated (Table XI).

One observes that the three records, based on two readings for the day, give differences that are greater or less than the "true" difference, and that on certain days these divergencies are so excessive as to discredit their use for the study of thermal influence in biology. It should also be stated that on a few days the differences were small. It is doubtful which record is most at variance with the thermograph record, thus indicating that none are worthy of consideration when careful analysis of climatic influence, from day

to day, is desired. It is worthy of note that this is the only comparison of the records that shows all three approximate methods of determining means to be equally at fault.

TABLE XI.— TEMPERATURE DIFFERENCE FROM DAY PRECEDING DATE, DURING MAY AND JUNE, 1916. FREDONIA, N. Y.

(Degrees Fahr.).

May.					June.				
Date.	Record.				Date.	Record.			
	Thermograph.	12 P. M.	8 P. M.	5 P. M.		Thermograph.	12 P. M.	8 P. M.	5 P. M.
1	21.0	18.5	20.5	11.5	1	11.9	13.5	15.5	16.5
2	.2	.5	3.0	11.0	2	12.2	14.5	7.5	5.5
3	6.1	5.0	4.5	4.0	3	4.2	5.5	1.5	4.5
4	6.6	9.5	9.0	9.5	4	1.4	2.0	1.0	.5
5	.3	4.0	3.5	3.0	5	1.2	.5	1.0	1.0
6	.2	3.0	5.5	5.0	6	2.6	2.5	.5	5.0
7	1.8	1.0	1.0	1.0	7	3.7	5.5	3.0	2.0
8	11.7	11.5	11.0	6.0	8	1.9	1.0	1.0	2.5
9	12.3	13.0	10.0	5.5	9	2.1	6.0	4.0	7.5
10	5.4	7.5	5.0	5.0	10	.3	2.0	2.0	.5
11	8.4	7.0	17.7	15.5	11	.9	0	0	1.5
12	6.9	4.0	4.0	2.0	12	4.6	3.0	3.0	3.0
13	2.8	3.5	3.5	4.0	13	3.9	6.0	6.0	6.0
14	12.9	4.0	15.5	12.5	14	3.3	4.5	4.5	0
15	8.8	7.0	10.0	3.0	15	3.7	3.5	3.0	1.5
16	15.1	15.5	13.0	16.5	16	4.2	3.0	2.0	.5
17	3.0	4.5	5.5	7.5	17	2.4	2.0	3.0	4.5
18	9.5	10.5	10.5	10.5	18	3.6	3.0	2.0	.5
19	2.3	2.0	2.0	.5	19	2.4	3.0	4.0	5.5
20	2.4	1.5	1.5	3.0	20	1.4	1.5	1.5	1.0
21	4.0	6.0	6.0	7.0	21	4.1	2.5	2.5	1.0
22	9.9	14.5	12.5	11.5	22	1.2	.5	1.0	1.0
23	1.3	.5	.5	1.0	23	10.3	15.5	16.0	15.0
24	1.2	1.5	.5	3.0	24	4.8	9.0	6.3	6.5
25	4.1	6.0	9.0	10.0	25	3.3	2.5	0	0
26	2.2	2.5	2.5	.5	26	2.8	3.5	5.0	6.0
27	2.3	4.5	5.5	5.5	27	6.3	4.0	5.5	5.0
28	0	2.0	2.5	2.5	28	.7	3.0	3.0	4.5
29	2.9	3.5	3.0	1.0	29	1.5	7.0	7.0	7.0
30	10.5	7.5	4.5	7.5	30	1.5	1.5	2.0	1.0
31	6.3	1.5	0	2.7					

EFFECT OF DIVERGENCES IN AVERAGES ON SUMMATION OF EFFECTIVE TEMPERATURE.

A favorite problem of phenologists has been the determination of the amount of heat-time units necessary to produce certain phenomena — especially blooming — in plants. Lately entomolo-

gists[16] have attempted the solution of a similar problem: the thermal-time value necessary to secure a certain activity or development in insects. Examples are emergence from hibernation and emergence of adults from the pupal stage. The solution of this problem, with certain species of insects, has a practical bearing on control measures. It is obvious that, if the methods are to give accurate predictions, one of the essentials is that the temperature data shall be correct.

The usual method of solution has been a summation of the daily-degree units above a certain temperature which has been assumed as the physiological zero of the species. When the zero point is not accurately known for the species under investigation, and especially when several species are under investigation at the same time, it has been customary to assume 39 or 41 degrees Fahrenheit as the temperature at, or below which, no activity will take place.[17] Taking 39 degrees as our physiological zero, and making a summation of the daily mean temperatures above this point, in all of the records for the months from April to August inclusive, we have found the values for each month, continuously, and have given the percentage of difference of each record from the summation using thermograph averages (Table XII). The summations were started on April 11 — since previous to this time the ground was covered with snow and ineffective temperature-degree units prevailed — and are to be considered as extending from that date to the end of the month indicated.

The summations from the three records are higher than that from the thermograph averages: that based on the five o'clock record being highest; the midnight lowest; and that based on the eight o'clock record occupying an intermediate position. The greatest discrepancies (percentage) occurred during April and May, and the percentages grow smaller as the summation extends farther into the summer. This would indicate that the summer months — summing each separately — gave smaller deviations from the true summation than did the spring months. In order to test this point, summations were made of the records of each month independently, using the same zero as before. The results are shown in Table XIII, and bear out the contention just mentioned.

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[16] Sanderson, E. D. The relation of temperature to the hibernation of insects. *J. Econ. Ent.* 1:56–65. 1908.

Sanderson, E. D. The relation of temperature to the growth of insects. *J. Econ. Ent.* 3:113–139. 1910.

In the second paper Sanderson has given an excellent discussion of the literature of effective temperatures in both botanical and zoological investigations and has appended a bibliography. The reader who desires to study the subject more extensively is referred to this paper.

[17] Kincer, J. B. Relation between vegetative and frostless periods. *Monthly Weather Review* 47:106–110. 1919.

In this paper is discussed a probable reason for the temperature point selected as physiological zero.

TABLE XII.— SUMMATION OF DAILY MEAN TEMPERATURES ABOVE 39 DEGREES FAHRENHEIT. FREDONIA, N. Y. 1916.
(From April 11 to end of month indicated).

Record	April		May		June		July		August	
	Summation	Per ct. discrepancy	Summation	Per ct. discrepancy	Summation	Per ct. discrepancy	Summation	Per ct. discrepancy	Summation	Per ct. discrepancy
Thermograph	151.7	0	654.9	0	1337	0	2474	0	3505	0
Midnight	164.5	8.4	672.5	2.7	1374	2.7	2523	1.9	3561	1.6
Eight P. M.	166.5	9.8	694.0	6.0	1410	5.5	2568	3.8	3613	3.1
Five P. M.	183.5	20.9	744.5	13.7	1477	10.5	2657	7.4	3721	6.1

TABLE XIII.— SUMMATION OF DAILY MEAN TEMPERATURES ABOVE 39 DEGREES FAHRENHEIT. FREDONIA, N. Y. 1916.
(Individual months).

Record	April		May		June		July		August	
	Summation	Per ct. discrepancy	Summation	Per ct. discrepancy	Summation	Per ct. discrepancy	Summation	Per ct. discrepancy	Summation	Per ct. discrepancy
Thermograph	151.7	0	503.2	0	682.1	0	1137	0	1031	0
Midnight	164.5	8.4	508.0	1.0	701.0	2.8	1149	1.0	1038	0.8
Eight P. M.	166.5	9.8	527.5	4.8	716.0	5.0	1158	1.8	1045	1.3
Five P. M.	183.5	20.9	561.0	11.5	732.5	7.4	1180	-3.8	1063	3.1

Inasmuch as phenological and entomological data are taken during the months when differences from the true values are large, might it not be possible that some of the variation in results, secured in different years, may be due to the fact that the means of maximum and minimum readings were used?[18]

EFFECT OF DIVERGENCES IN AVERAGES ON THE SUMMATION OF TEMPERATURE COEFFICIENTS.

The Livingstons[19] have introduced a modified summation method, in which they consider that growth and development of plants, to a certain extent at least, are chemical in their nature, or else depend on reactions that are chemical, and that the rate of growth practically doubles for each increase of eighteen degrees Fahrenheit. Assuming that the principle of van't Hoff and Arrhenius holds generally in plant growth, they have calculated "approximate efficiency indices" from the formula

$$u = 2^{\frac{t-40}{18}}$$

in which t is taken as the daily mean temperature and u represents the index to be found. These have been computed (in whole degrees) from 40 to 99 degrees Fahrenheit and recorded in a table.[20] Thus 40 degrees has an efficiency value of 1; 58 degrees, 2; and 76 degrees, 4. Using the values in Livingstons' table (interpolating for fractions of a degree), the index for each day from April 11 to August 31 has been determined for the four records, and the summations made. These are shown, for the continuous summations, in Table XIV, and, for the individual months, in Table XV.

A noticeable feature of the summation of indices as compared with the summation of temperatures, is the smaller values in the percentage of discrepancy in every record during April, May and June when the Livingston coefficients are used, while these values are similar during July and August. As regards accuracy, the three approximate records show the same differences as have been noted thruout the bulletin.

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[18] The writer is not making a defence of any form of summation for comparison with activities of plants and animals at certain definite points in the march of seasonal phenomena. His effort is aimed to show how the methods of computation of daily averages may influence the results of summation.

[19] Livingston, B. E. and Livingston, G. J. Temperature coefficients in plant geography and climatology. *Bot. Gaz.* 56:349–375. 1913.

[20] Loc. cit., p. 366.

TABLE XIV.— SUMMATION OF LIVINGSTON TEMPERATURE INDICES. FREDONIA, N. Y. 1916.
(From April 11 to end of month indicated).

Record	April		May		June		July		August	
	Summation	Per ct. discrepancy	Summation	Per ct. discrepancy	Summation	Per ct. discrepancy	Summation	Per ct. discrepancy	Summation	Per ct. discrepancy
Thermograph	27.6241	0	88.0565	0	161.2101	0	291.0265	0	406.2813	0
Midnight	28.1944	2.1	88.9777	1.0	164.0902	1.8	295.7198	1.6	412.3229	1.5
Eight P. M.	28.3599	2.7	90.7410	3.0	167.1987	3.7	300.3530	3.2	417.8335	2.8
Five P. M.	29.4089	6.5	94.2440	7.0	172.4793	7.0	309.7890	6.4	429.8532	5.8

TABLE XV.— SUMMATION OF LIVINGSTON TEMPERATURE INDICES. FREDONIA, N. Y. 1916.
(Individual months).

Record	April		May		June		July		August	
	Summation	Per ct. discrepancy	Summation	Per ct. discrepancy	Summation	Per ct. discrepancy	Summation	Per ct. discrepancy	Summation	Per ct. discrepancy
Thermograph	27.6241	0	60.4324	0	73.1586	0	129.8164	0	115.2548	0
Midnight	28.1944	2.1	60.7833	0.6	75.1125	2.7	131.6296	1.4	116.6031	1.2
Eight P. M.	28.3599	2.7	62.3811	3.2	76.4577	4.5	133.1543	2.6	117.4805	2.0
Five P. M.	29.4089	6.5	64.8351	7.3	78.2353	7.0	137.3097	5.8	120.0642	4.2

CONCLUSIONS.

In these investigations, no record based on maximum and minimum temperatures was found as accurate as the thermograph average, and the nearer to midnight these readings were taken the closer did the daily averages approximate the truth.

The annual mean temperature, calculated from records taken at 8 P. M. or later, gave differences so slight as to be negligible, and, for this reason, the records of the coöperative observers of the U. S. Weather Bureau are reliable for biological workers, so far as the annual mean temperature is concerned.

Monthly mean temperatures, computed from the highest and lowest temperatures for each twenty-four hours when the hour of observation occurs not earlier than 8 P. M., while safe for climatological purposes, may introduce errors of importance to ecological workers, and so recourse should be had to temperature curves in order to determine the discrepancies.

Perhaps the main point of this study lies in the fact that it has proved that averages to be compared should be calculated from readings taken at the same hour, otherwise erroneous conclusions may be drawn, thus corroborating the studies of Mr. C. A. Donnel referred to in footnote on page 399.

The taking of readings before eight o'clock in the afternoon is not to be recommended for any purpose, owing to the extreme differences that will be introduced in the averages.

Daily mean temperatures determined by approximate methods are practically worthless for exact comparisons of temperature data from day to day as is demanded by zoölogical and botanical investigations under natural conditions. Also these means are the cause of errors of considerable magnitude in the summation of temperatures or indices, especially the former, during the spring months.

To secure accurate averages of all kinds the ecological worker should rely upon thermograph records, which have been checked and, if necessary, corrected from readings of maximum and minimum thermometers of precision.

REPORT

OF THE

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II. Twenty years of fertilizers in an apple orchard.

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REPORT OF THE DEPARTMENT OF HORTICULTURE.

A TEST OF COMMERCIAL FERTILIZERS FOR GRAPES.

FRED E. GLADWIN.

SUMMARY.

These tests show that nitrogen, phosphorus and potassium have had a marked beneficial effect upon wood growth, yield and quality of fruit. The data indicate that of the three elements nitrogen has been most helpful.

Potassium has given more pronounced results than phosphorus up to the present, altho the latter has had a more beneficial effect upon the green-manure crops in the vineyard.

Nitrogen has not only affected favorably the growth of wood but it has increased the fruit and given larger berries and clusters. Phosphorus and potassium have increased the production of wood and fruit, but have not influenced the quality of the fruit to the same extent as the nitrogen. Potassium has caused earlier ripening of the foliage than the other elements.

Even tho the same number of canes be tied up for fruiting purposes, the data show that the fertilizer plats have produced a decided gain of fruit over the unfertilized.

The foliage, after the first few years, has been of better color and size in the plats to which nitrogen was applied. That from the phosphorus and potassium plats ranked second, with that from the check a poor third.

INTRODUCTION.

In the spring of 1914 this Station published in Bulletin No. 381 the results of a five-year test of commercial fertilizers on grapes. The following conclusions, a part of the summary of that work, are as applicable now as then:

1. It is evident that the fertilization of vineyards is so involved with other factors that only long-continued work will give reliable results.

2. Fertilizers cannot be profitably applied in vineyards poorly drained, those subject to unseasonable cold, or in those in which fungi or insects are uncontrolled, or where good culture is lacking.

* **Reprint of Bulletin No. 458, January, 1919.**

3. It is probable that most vineyards have a one-sided wear, there being few plantations where more than one or two of the elements of fertility are lacking. Nitrogen is probably the element most frequently needed.

4. The steps to be taken in restoring a failing vineyard are, in the order of importance: 1. Drain the land. 2. Control insects and fungi. 3. Improve tillage and general care. 4. Apply such fertilizers as may be found lacking.

Data are here set forth for another five-year period from the same vineyard, a continuation of the test. It is necessary to describe again the experiment and its environment.

The Experiment.

Approximately three acres were selected in 1909 for the test of commercial fertilizers. This area is uniform and has a gentle slope to the south. A slight depression extends across the section from west to east. The plats extend at right angles to this depression, so that the topography is fairly uniform. The soil at the north end of the vineyard is a little lighter in texture than elsewhere in the section, but nearly equal amounts of each plat overrun this variation. The rows, 46 in number, run north and south, and each contains 37 vines. The vines had been set 18 years when the experiment began. At this time it was a representative vineyard for this type of soil, except that the west side, including about 20 rows, was in poorer condition than the remainder. Plats 1, 2 and 3 fall in this poorer part.

As far as could be learned, no commercial fertilizer nor stable manure had been applied to this section for at least ten years previous to the beginning of the experiment. The tillage had been that ordinarily given; namely, spring plowing, horse-hoeing, hand-hoeing and subsequent cultivation with spring-tooth and disc harrows.

The section was divided into 11 plats consisting of three rows each with a buffer row separating adjacent plats. The plats are numbered from one to eleven in order from west to east.

The vines are planted in rows eight feet apart and at intervals of eight feet in the row. Thus, there are six hundred eighty vines to the acre. Were all the vines in, each plat would include one hundred eleven vines, or approximately one-sixth of an acre.

Fertilizers were applied annually as follows:

Plats 1 and 7.

Nitrate of soda at the rate of 100 pounds per acre.
Cottonseed meal at the rate of 800 pounds per acre.
Acid phosphate at the rate of 300 pounds per acre.
Sulphate of potash at the rate of 200 pounds per acre.
Lime, every third year, at the rate of 2,000 pounds per acre.

Plats 2 and 8.

The same as for plats 1 and 7, less the lime application.

Plats 3 and 9.

Nitrate of soda at the rate of 100 pounds per acre.
Cottonseed meal at the rate of 800 pounds per acre.
Acid phosphate at the rate of 300 pounds per acre.

Plats 4 and 10.

Nitrate of soda at the rate of 100 pounds per acre.
Cottonseed meal at the rate of 800 pounds per acre.
Sulphate of potash 200 lbs. per acre.

Plats 5 and 11.

Sulphate of potash at the rate of 200 pounds per acre.
Acid phosphate at the rate of 300 pounds per acre.

Plat 6. Unfertilized.

TABLE I.— PRICE PER TON OF COMMERCIAL FERTILIZERS USED IN GRAPE FERTILIZER EXPERIMENTS.

Commercial fertilizers.	1909.	1910.	1911.	1912.	1913.	1914.	1915.	1916.	1917.	1918.
Nitrate of soda	$54 00	$49 25	$49 00	$50 00	$56 00	$50 00	$54 00	$78 00	$75 26	$100 00
Dried blood	39 50	40 00	55 00	55 00	40 00	45 00	49 00	50 00	73 57	....
Cottonseed meal	24 75	......	......	......	......	......	......	......	......	....
Acid phosphate	13 00	13 00	13 00	13 00	11 00	10 00	14 00	17 00	.....	19 20
Sulphate of potash	47 00	45 00	48 00	48 50	45 00	47 00	......	......	.....	....
Lime	6 00	......	......	6 00	......	......	7 00	......	.....	....

After the first year, dried blood was substituted for the cottonseed meal owing to the difficulty of obtaining the latter. The amount of dried blood used in 1910 was at the rate of 560 pounds per acre. The same amount of nitrogen was applied in either case. In 1918, owing to the difficulty of obtaining dried blood, nitrate of soda was used as the only nitrogen carrier, at the rate of 250 pounds per acre. Potash could not be obtained in 1918. Applications of lime have been made at three-year intervals. Table I gives the prices paid for fertilizers.

In 1909, the cottonseed meal and nitrate of soda were mixed with the other materials, excepting the lime, and immediately spread and plowed under. In the following years, the dried blood and nitrate of soda were withheld from the mixtures, and two separate applications of them made, the first shortly after the leaves began to unfold, and a second two or three weeks later. The applications of the nitrogen were broadcasted on the surface and lightly harrowed in. The acid phosphate and sulphate of potash were applied before spring plowing, so that they were turned down to a considerable depth. When the vineyard was plowed in the fall, these materials were applied.

Cover Crops.

Cover crops have been grown yearly, the following crops having been sown: rye, barley, cow-horn turnip, winter wheat and dwarf Essex rape. Of all these, the most satisfactory growth has been secured from the rye. The time of seeding was governed by the amount of soil moisture present, ranging from July 24th to August 15th.

Cultivation.

The tillage has been that practiced by successful growers, consisting of plowing with either a three-gang vineyard, two-horse, or single-horse plow; immediately after this the disc harrow has been used to cut the turned cover crop, and at the same time to bring the soil back to a level; following the discing, the spring-tooth harrow has been used thruout the summer. The aim has been to preserve a fine dust-mulch thruout the growing season, hence the number of cultivations has been dependent upon the rainfall. Previous to the seeding of the cover crops, the rows have been plowed once around up to the vines with the single-horse plow, leaving the middle of the rows almost level over winter. For the past five years, the spring plowing has been up to the vines.

Pruning and Training.

The Chautauqua method of training was practiced in pruning. The vines were pruned upon their performance of the previous season, as judged by the amount of fruit and wood produced. Except in a few instances, the number of canes tied up has not exceeded four per vine, in many years the average being less than this number, depending on the length of the internodes.

Spraying.

In the first years of the experiment the vineyard was sprayed twice each season, but as the root-worm, the worst pest, was brought under control, it was seen that one application was sufficient. In the ten years, two sprayings have been made for the grape leafhopper.

Weather Conditions.

The crop in 1909, the year the experiment began, was large. Vineyardists were of the opinion that the era of low yields was past. The growth of vine was all that could be desired as judged during the summer, but when the time for pruning came it was evident that, while growth was ample, it was not well ripened. In the spring of 1910 it was found that fully fifty per cent of the buds retained to carry the crop of 1910 failed to start, the opinion prevailing that late spring frosts had killed them. More likely, however, the injury came indirectly from the heavy crop of 1909, which so delayed the maturity of the buds that they were killed by the cold of the following winter.

Winter of 1911-1912.

The 1911 yield was even larger than that of 1909. The fall and winter temperatures following this large crop were characterized by frequent and decided changes of temperature, January, 1912, being the coldest on record, —15° and —19° being recorded. The cold weather continued until the middle of February, and after that another severe cold period occurred in March. The soil froze to unusual depths. The grape crop in 1912 in old vineyards fell considerably below that of 1911. The wood growth for the season was very scant, and examination disclosed many injured buds.

Winter of 1915–1916.

Weather records show that the growing and maturing periods of 1915 were decidedly unfavorable for the vine, and, while the lack of maturity of wood growth was not so evident as in 1909 and 1912, yet 18 per cent. of Concord buds were killed outright during the winter of 1915–1916, while many of those that started later failed to function, with a consequent decrease of the normal number of fruit clusters.

Winter of 1917–1918.

The season of 1918 marks the lowest yield in the history of the Chautauqua Belt. This low production was directly influenced by the lack of maturity reached by fruit and wood in the season of 1917, coupled with the cold of the winter following. This improper maturity was due to the weather during the months of September, October and November. At the beginning of September, the season was fully two weeks later than the average, this being due in large part to the backward spring. September was one of the coolest on record, with half the days cloudy or partly cloudy tho the rainfall was below normal. October, with one exception, was the coldest in the history of the weather bureau, and a new record was established for moisture, 8.86 inches being recorded at the Station grounds. Rain fell on 19 days of the month, while 27 days were cloudy or partly cloudy. Killing frosts occurred October 9th, 10th and 11th. The grape harvest was completed November 6th. In the first six days of November, 0.50 inch of rain and 8.1 inches of snow fell.

Sudden changes from high to low temperatures characterized the months of December, January and February. The season was notable also for frequent high winds and gales.

From the above statements, it is seen that the yield has been decreased in four of the ten seasons by weather conditions.

Gauging Results.

In the first two years, 1909 and 1910, records were made of the fruit yields from the different plats and of the vigor of the vines as indicated by the wood growth and the amount and color of the foliage. In the last eight years, the weight of the pruned wood, the amount of fruiting wood put up and the average number of buds per cane, also, have been recorded for each plat.

Yield of Fruit.

In Table II the yields for ten years are given. The yield in this vineyard previous to 1909 is not known, but the owner was dissatisfied with the vineyard because of its poor condition, especially that portion on the west which includes Plats 1 and 2. The table shows that in 1909 the unfertilized plat yielded less than any other

TABLE II. — YIELD OF GRAPES (IN TONS PER ACRE) ANNUALLY FOR THE PAST TEN YEARS, WITH THE TEN-YEAR AVERAGE FROM FERTILIZER EXPERIMENT.

TREATMENT.	1909.	1910.	1911.	1912.	1913.	1914.	1915.	1916.	1917.	1918.	10-year average.
	Tons.	*Tons.*	*Tons.*	*Tons.*	*Tons.*	*Tons.*	*Tons.*	*Tons.*	*Tons.*	*Tons.*	*Tons.*
1. Complete fertilizer-lime	4.48	2.10	5.37	3.46	2.14	4.90	2.55	1.60	3.35	1.19	3.11
2. Complete fertilizer	4.76	2.21	5.71	4.30	2.83	5.20	2.78	1.70	3.46	1.45	3.44
3. Nitrogen-phosphorus	5.17	2.14	5.61	4.00	2.25	4.00	2.70	2.10	3.00	1.05	3.20
4. Nitrogen-potassium	4.25	2.55	5.64	4.10	2.85	5.30	3.20	1.40	3.60	1.19	3.40
5. Phosphorus-potassium	3.41	2.00	5.44	4.35	1.78	4.00	2.90	1.70	3.30	.76	2.96
6. Check	3.38	2.10	5.32	3.60	1.24	2.90	2.89	1.30	2.60	.45	2.58
7. Complete fertilizer-lime	4.69	2.38	5.62	4.80	3.04	5.10	3.50	2.50	3.60	1.45	3.67
8. Complete fertilizer	4.66	2.07	5.71	4.98	2.72	5.80	3.70	2.20	4.00	1.38	3.72
9. Nitrogen-phosphorus	4.99	2.04	5.35	4.89	2.61	4.80	3.90	2.10	3.50	1.49	3.57
10. Nitrogen-potassium	4.79	2.26	5.91	4.89	3.07	5.70	3.70	2.20	4.47	1.25	4.02
11. Phosphorus-potassium	4.99	1.87	5.03	4.21	1.97	4.50	3.67	1.90	3.57	.97	3.27

plat in the vineyard. Differences in wood growth or in the color of the foliage were not discernible in any of the plats. There was an even production over all the plats in 1910, which may have been influenced by the winter killing of 50 per cent of all buds irrespective of fertilizer treatment. The yield over all the plats in 1911 was as uniformly high as they were low in 1910.

That certain fertilizers contribute to higher yields, even when the amount of fruiting wood is not greater than that on unfertilized vines, is evident from a comparison of Table II with Table III, which shows the number of fruiting canes left annually in the different plats. In 1912, it is seen in Table III that the check plat, and the second complete fertilizer-lime plat had the same number of fruiting canes, namely, 2,856. From Table II it is seen that the complete fertilizer-lime plat yielded 1.2 tons more per acre than the check. This can be accounted for only by the greater vigor of the fertilized vines. It is worthy of note that each fertilized plat, except No. 1, complete fertilizer-lime, yielded higher than the check.

In 1913, the check plat is the lowest producer. The differences between it and the fertilized plats range from 0.54 ton in the case of Plat 5, phosphorus and potassium, to 1.83 tons with Plat 10, nitrogen and potassium. In this year both phosphorus and potassium plats, which up to 1913 produced crops comparable with any of the others, gave lower yields than any other of the fertilized plats.

Table II shows that the check plat fell far below all others in 1914. The increases from the fertilizers range from 1.1 tons to 2.8 tons per acre. The phosphorus and potassium plats this season again show a tendency to equal those to which nitrogen was applied. In 1915 the plats east of the check returned increased crops over the latter, while but one to the west of it did so. With the years following, 1916–1917 and 1918, the table shows that the check has without exception been the low producer.

The evidence presented in Table II seems to confirm an earlier statement that the west part of this vineyard was in poorer condition, either thru a lack of fertility or from the depredations of insects and disease, than that part to the east of the check.

With the ten-year averages at hand, it is seen that, in spite of the fluctuations from year to year, every fertilized plat has produced an increase over the check.

TABLE III.— TOTAL NUMBER OF FRUITING CANES PER ACRE LEFT ANNUALLY ON GRAPE VINES IN FERTILIZER EXPERIMENT PLATS.

TREATMENT.	1912.	1913.	1914.	1915.	1916.	1917.	1918.	7-year average.
1. Complete fertilizer-lime	2,815	1,985	2,332	1,917	1,795	1,985	2,067	2,128
2. Complete fertilizer	3,033	2,373	2,366	2,046	1,999	2,148	2,210	2,325
3. Nitrogen and phosphorus	3,094	2,373	2,142	2,040	1,781	1,999	1,856	2,185
4. Nitrogen and potassium	3,039	2,407	2,454	2,176	2,155	2,223	2,230	2,385
5. Phosphorus and potassium	3,019	2,121	2,033	1,985	1,992	1,999	1,952	2,156
6. Check	2,856	2,087	1,387	1,842	1,768	1,795	1,537	1,896
7. Complete fertilizer-lime	2,856	2,400	2,386	2,210	2,400	2,182	2,122	2,365
8. Complete fertilizer	3,066	2,407	2,407	2,318	2,318	2,414	2,278	2,458
9. Nitrogen and phosphorus	3,289	2,366	2,244	2,210	2,189	2,257	2,285	2,405
10. Nitrogen and potassium	3,107	2,434	2,434	2,312	2,155	2,407	2,278	2,447
11. Phosphorus and potassium	2,862	1,897	2,019	2,094	2,002	2,040	2,054	2,138

A comparison of the yields of the check plat with the two adjacent plats perhaps is more valuable than one including all the plats. These three plats, consisting of nine rows with buffer rows between, separate the section into two equal parts, while the topography is most uniform. From a study of these figures thru each year and the ten-year average, one is able to judge the relative values of nitrogen, phosphorus and potasssium for the vine. The lime included with the complete fertilizer can be ignored, as is shown by a comparison with the complete fertilizer plat immediately adjoining. The ten-year average of these three plats shows that the phosphorus and potassium plat yielded an average of 0.38 tons more per acre each year over the ten years than did the check, while the complete fertilizer-lime plat on the other side of the check has yielded a yearly average of 1.09 tons more for the same period.

Effects on the Fruit.

As stated in Bulletin 381, no differences in fruit characters were to be noted in the various plats up to 1912. At the harvest of this year, and in each year following, the fruit from all fertilized plats was superior in compactness of cluster, size of cluster and size of berry. This has been more marked during the past five seasons than at any time previous, with the greatest differences occurring in 1918. During the first few years of this experiment, the fertilized plats matured their fruit earlier. This has not been so evident in later years except in the phosphorus and potassium plats. The clusters in the phosphorus and potassium plats, while markedly superior to those of the check, were not the equal of those from the plats to which nitrogen had been applied. The clusters from the check have run small and loose, with berries below the average in size. In 1918, the fruit from the check plat was greatly inferior to any from the fertilized. While the sugar content ran higher with the check than all others, yet the general appearance of the fruit was such that it could not compete in the market with that from the fertilized plats. Under ordinary conditions a light crop is correlated with compactness of cluster, larger size of cluster and larger berries, while a heavy yield results in the converse. The check vines, without the intervention of other factors, should have yielded fruit of superior characters.

TABLE IV.— COMPARATIVE WEIGHTS OF PRUNED WOOD PER ACRE FROM GRAPE VINES IN FERTILIZER EXPERIMENT.

TREATMENT.	1911.	1912.	1913.	1914.	1915.	1916.	1917.	1918.	Average 1911–1918.
	Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
1. Complete fertilizer-lime	*1,244	1,020	1,088	1,033	†686	993	993	904	995
2. Complete fertilizer	*1,387	1,196	1,292	1,194	†850	1,129	1,142	1,088	1,159
3. Nitrogen and phosphorus	*1,360	1,156	1,088	972	†721	864	864	680	966
4. Nitrogen and potassium	*1,360	1,258	1,360	1,217	†864	1,163	1,020	707	1,117
5. Phosphorus and potassium	*1,224	1,033	816	972	†687	959	816	510	877
6. Check	1,305	707	734	761	†520	680	666	313	711
7. Complete fertilizer-lime	1,734	1,162	1,496	1,278	†1,122	1,272	952	911	1,241
8. Complete fertilizer	1,747	1,183	1,400	1,339	†1,278	1,387	1,224	856	1,302
9. Nitrogen and phosphorus	1,679	1,162	1,190	1,142	†1,088	1,142	1,020	884	1,163
10. Nitrogen and potassium	1,720	1,203	1,407	1,258	†1,238	1,183	1,224	802	1,254
11. Phosphorus and potassium	1,489	1,003	952	938	†938	986	1,020	721	1,006

* Weights taken in spring of 1912; the pruning was done in the fall preceding.

† Weights taken in March, 1916, the pruning having been done in December, 1915.

Effects on the Vines.

For the first three years, there were no indications of differences in the amount or color of the foliage in the different plats. One unfamiliar with the experiment could not have selected any one plat as being superior to any other. Since 1912, however, all the plats fertilized with nitrogen have shown more abundant foliage, which has been of better color, and the check has shown the poorest foliage. While the foliage of the phosphorus and potassium plats has been somewhat superior to that of the check, yet the amount and color has not been comparable to that of the nitrogen plats. The effect of the potassium in the phosphorus and potassium plats in its ripening influence on leaves was striking in 1917 and even more pronounced in 1918. The two latter plats had distinctly yellowish foliage, while the nitrogen plats were still a vivid green.

Annual Wood Growth.

Table IV records the amount of wood pruned annually from the plats under discussion, beginning with 1911, with the average for eight years. After two years' efforts to gauge the effects of the fertilizers on wood growth, it was decided that weighing the pruned wood would best answer the purpose. So far as the eye could see, there were no indications that the fertilizers were producing any increases in cane growth.

Table V gives the average amount of wood pruned per acre and the average number of canes tied up per acre for each plat for the seven years. With these data at hand, the effects of fertilizers in promoting cane growth become apparent. The check plat has developed the least amount of wood of any of the plats, and the phosphorus and potassium plat adjacent, while it has produced more growth than the check, yet falls far short of that produced in the complete fertilizer-lime plat located at the other side of the check. Again, as with fruit yields, the plats on the west side of the section are inferior to the plats situated to the east receiving similar treatment. Examination of Table V also indicates that while the check plat has had less wood pruned from it, it has also had a less number of canes put up each year. With one exception, complete fertilizer-lime Plat No. 1, the phosphorus and potassium plats have had fewer canes tied up over the entire period than the plats that have had nitrogen applications. Nevertheless, while nitrogen exerts

TABLE V.— COMPARATIVE WOOD GROWTH OF GRAPE VINES WITH NUMBER OF FRUITING CANES PUT UP IN FERTILIZER EXPERIMENTS.

TREATMENT.	Average amount of wood pruned per acre from 1911 to 1918 inclusive.	Average number of canes put up per acre from 1911 to 1918 inclusive.
1. Complete fertilizer-lime	1,005	2,128
2. Complete fertilizer	1,170	2,325
3. Nitrogen and phosphorus	1,003	2,183
4. Nitrogen and potassium	1,178	2,385
5. Phosphorus and potassium	929	2,156
6. Check	810	1,896
7. Complete fertilizer-lime	1,289	2,365
8. Complete fertilizer	1,365	2,458
9. Nitrogen and phosphorus	1,203	2,405
10. Nitrogen and potassium	1,319	2,447
11. Phosphorus and potassium	1,047	2,138

the greatest influence in increasing wood growth in this experiment, it would seem from the data that potassium is helpful also.

Financial Gain or Loss for the Ten Years.

In Table VI the gain or loss per acre for the several plats is given. The price paid for the various fertilizer carriers has varied from year to year, as has the selling price of the fruit. No charge for freight, cartage or application has been included as these will vary with location, methods of cartage and mode of application. These charges have ranged from $3.20 per acre for the complete fertilizer-lime plats in 1909 to $2.99 in 1918, while for the potassium and phosphorus plats the charge ranges from $1.55 in 1909 to $2.17 in 1918.

The figures up to 1914 are actual prices paid for the various carriers. As the opening of the war greatly increased fertilizer costs, especially all forms of potash, nitrate of soda and dried blood, the prices paid in 1914 have formed the basis of the computations for the succeeding years.

From Table VI it is evident that fertilizers have paid a good return on the investment in all the plats under experiment. Plat I, complete fertilizer-lime, is as it was at the beginning, the low producer, but, with all the ups and downs, it shows a total gain of $72 for the ten-year period.

A comparison of Plats 5 and 7, situated one on either side of the unfertilized check, is interesting. The differences in treatment between the two are that Plat 7 has had a nitrogen application as well as lime in addition to the elements applied to Plat 5. Lime is of doubtful value for the vine, with some evidence that it may be actually harmful; hence in this comparison it is negligible. Plat 5, phosphorus and potassium, shows a total net gain for the ten-year period of $121.52, while Plat 7, complete fertilizer-lime, has returned a net of $303.43, or a gain of $181.91 over Plat 5. Likewise if the total gain of Plat 5, phosphorus and potassium, be deducted from the totals from Plats 1, 2, 3 and 4, it will be seen that, with the exception of Plat 1, there has been a clear gain of money above Plat 5. This gain can be ascribed only to the addition of nitrogen to the formula. The differences range from $32 to $101.

Again, if the total gain from Plat 11, phosphorus and potassium, be deducted from the totals of Plats 8, 9 and 10, it is apparent that

TABLE VI.— FINANCIAL GAIN OR LOSS PER ACRE FROM THE USE OF FERTILIZERS ON GRAPES WHEN COMPARED WITH THE MONEY RETURN FROM THE UNFERTILIZED CHECK PLAT.

TREATMENT.	1909.	1910.	1911.	1912.	1913.	1914.	1915.	1916.	1917.	1918.	Total gain, —10 years.
1. Complete fertilizer; lime	$8 92	—$18 91	—$18 08	—$27 21	$26 05	$49 30	—$20 70	—$6 00	$21 64	$57 00	$72 01
2. Complete fertilizer	18 63	—11 91	—7 75	4 17	62 55	62 80	—17 70	1 90	32 18	87 30	231 17
3. Nitrogen and phosphorus	34 65	—10 59	—5 99	—1 41	38 05	25 50	—13 00	26 20	10 20	50 00	153 61
4. Nitrogen and potassium	6 55	5 51	—7 93	—86	65 20	67 80	—5 66	—11 30	41 80	61 50	222 61
5. Phosphorus and potassium	—7 52	—11 00	—2 50	19 44	20 85	32 30	—6 20	13 40	36 40	26 35	121 52
7. Complete fertilizer; lime	14 70	—6 17	—11 88	19 69	71 05	56 30	04	38 10	37 30	84 30	303 43
8. Complete fertilizer	15 88	—18 27	—9 98	27 99	57 05	83 80	9 84	31 30	63 50	79 95	341 06
9. Nitrogen and phosphorus	29 70	—15 14	—14 49	29 70	56 05	53 50	21 34	26 20	39 20	96 20	322 26
10. Nitrogen and potassium	21 40	—7 68	27	26 79	76 20	81 80	11 34	27 90	92 26	67 80	398 18
11. Phosphorus and potassium	37 57	—16 91	—14 95	14 54	30 35	49 80	20 32	23 20	50 05	48 40	242 38

the latter treatments have returned from $79 to $155 more per acre than the amount derived from Plat 11, an average of $111 for the three plats, 8, 9 and 10.

Considering the money returns from the two sets of figures just given, it appears that the nitrogen applied either in conjunction with phosphorus or potassium, or with both, is worth something more than $100 per acre for the ten-year period.

Table VI further indicates that the phosphorus and potassium combination has proved highly profitable, and that of these two elements, the potassium seems to have been the more effective. That phosphorus applications have been of value has been demonstrated from its effects upon the green-manure crops grown in this vineyard. It has affected, as has no other of the materials, the growth of rye, barley, wheat and cow-horn turnips. It is believed that, through the promotion of better growths of green manures, phosphorus will, in the end, contribute in a measurable degree to the production of fruit and wood.

Suggestions.

The fact that two most unusual seasons have just been passed does not imply that the climate of this or any other part of the State is undergoing a permanent change for the worse. It does, however, emphasize the fact that the vigor of the average vineyard in the Chautauqua Belt has not materially increased during the past ten years. The degree of injury from the rigors of the winter of 1917–1918 coincides quite closely with age of vine and the conditions under which it was growing. Those already weakened from insects, lack of fertility and poor cultural care were the ones to suffer the greatest injury. It must not be assumed that, had the above conditions been met, there would have occurred no injury, for exposure to prevailing winds and other factors are involved. Newly planted vines that had not been taxed in the production of large crops, nor had as yet been seriously injured by insect depredations, nor had depleted the soil of the principal elements of fertility, suffered the least. In spite of reasonably good care, the Station lost many vines outright the past season, but such losses were invariably from plats or areas that have received no fertilizers or manure.

The chief suggestion to be made is to reiterate the statement that, before applying fertilizers to vineyards, one should first attend

to the fundamentals of good care, drainage, cultivation, cover crops, protection from insects and diseases, and proper pruning. If after this the condition and production of the vines are still unsatisfactory, fertilizers should be applied. In the absence of more definite knowledge, it can only be suggested that a fertilizer containing a rather large percentage of nitrogen be used.

TWENTY YEARS OF FERTILIZERS IN AN APPLE ORCHARD.

U. P. HEDRICK AND R. D. ANTHONY.

SUMMARY.

In Bulletin 339 of this Station, published in 1911, the results are presented for the first seven harvests in a fertilizer experiment which was begun with the planting of the trees on the Station grounds in 1896. The conclusions reached at that time can be condensed to a single statement — " the trees in this experiment would have been practically as well off had not an ounce of fertilizer been applied to them." The present bulletin discusses the results secured in eight additional harvests.

With the exception of a few of the early years, the orchard has been cultivated annually, and a non-leguminous cover-crop sown. The land is probably not as fertile now as at the beginning of the experiment, but there have never been noticeable differences in the cover-crop on the various plats.

The first crop was borne in 1902. Since 1910 the crops have been large and fairly regular. In 1918 the trees averaged over thirteen bushels.

The factors considered in interpreting results are yield and size of fruit and tree growth.

There are at least three factors causing lack of uniformity in tree performance in the orchard. A depression in the southwest corner has seriously decreased the yield and growth of Plat 7 and, to a less extent, Plat 8. A clay area in the southeast quarter has made Plats 11 and 12 ununiform in yield, and an area of lighter soil has probably decreased the yields of two trees in Plats 2 and 3.

Because of these variations, it does not seem wise to compare the duplicate plats; instead, the results are usually presented by plats as they stand in the orchard, the tables being diagrams of the orchard.

* Reprint of Bulletin No. 460, May, 1919.

In nearly all cases the result given in the tables is the average of the five trees in the plat. The probable error of the mean is given in order to indicate the degree of confidence which may be placed in this average.

Only summary tables are included in the body of the bulletin. The complete tables are in the appendix.

The average yield of the five trees in each plat for the total of fifteen harvests and the relative rank of each plat are given in Table III.

Adding acid phosphate at the rate of 340 pounds per acre per year has not given a noticeable increase in yield.

The addition of 196 pounds of muriate of potash to the 340 pounds of acid phosphate seems to have resulted in an increased yield.

The annual application of 50 pounds of readily available nitrogen in addition to the phosphoric acid and potash has caused no increase in yield.

Plats receiving stable manure have yielded no more than the check plats.

In general there are so many inconclusive or contradictory results that no conclusion of practical value can be drawn from the yields.

When we compare the rank in yield of the plats for the period ending in 1910 with the rank for the last eight years we see a tendency for the checks and phosphoric acid plats to take a slightly lower rank as the experiment has continued.

The average percentage of fruit grading two and one-half inches or larger is given for each plat. There is a greater difference between two nearby check plats than between any fertilized plat and its nearest check. This, together with the variations among the duplicates of the fertilizer treatments, makes it impossible to draw any definite conclusion as to the effect of treatment upon size of fruit.

The average trunk diameter and the approximate average tree volume for each plat are excellent factors to use in comparing the various plats. The two phosphorus and potassium plats lead their adjoining plats both in size of trunk and in tree volume, but it is not possible to say whether the increases are due to the potassium or the combination of the two elements, or to some tree or field variation which does not now show.

Heavy applications of nitrogen in a complete fertilizer and in manure have not increased tree growth.

When the costs are considered, certain plats have given increases sufficient to equal the costs, or even to show a profit, but in other plats the same plant food elements have shown a financial loss.

If the results continue in the present direction for another ten years, the increased yields may justify the recommendation of one or two of the treatments, but at present this cannot be done.

These results are from a cultivated orchard on soil naturally well supplied with the plant food elements. On thin, infertile soils or in sod orchards, the results might be quite different.

HISTORY AND LOCATION OF THE EXPERIMENT.

In the spring of 1896, an orchard of Ben Davis trees especially selected for uniformity was planted on one of the Station fields. That summer the trees were top-budded to Rome, all the buds coming from a single bearing tree in one of the Station orchards. The trees were planted 40 feet apart in the plats, and the plats 80 feet apart. Buffer rows of mixed varieties were planted in between, so that all trees stood 40 feet apart.

The field is nearly level except for a slight depression in the southwest corner. The soil is a heavy clay loam, and, being very retentive of moisture, was tile drained when the orchard was planted. Because of the amount of clay present and the lack of humus, the soil needs to be worked at the most favorable time in order to keep it in good physical condition. Chemical analyses indicate that it is well supplied with phosphorus and potassium.

A study of this field and its previous history when planted to field crops led those in charge of the work at that time to believe the soil sufficiently uniform for a fertilizer experiment, and it was with this point in mind that the trees were top-budded to Rome. For the fertilizer experiment the Romes were divided into twelve plats of five trees each, as shown in Figure 34. Five different treatments were decided upon: stable manure, complete fertilizer, phosphoric acid and potash, phosphoric acid alone, and check plats receiving nothing. The orchard was divided into quarters, and the diagonally opposite corners are duplicates. There are four check plats, one in each quarter, and two each of the other treatments, one in the north half and one in the south half of the orchard.

TABLE I.— AMOUNTS OF MATERIALS ANNUALLY APPLIED IN ORCHARD FERTILIZER EXPERIMENT.

TREATMENT.	Plats.	Pounds per acre.	PLANT FOOD PER ACRE.		
			N.	P_2O_5.	K_2O.
Checks	3, 5, 7, 11	....	....	....	.....
Stable manure	1, 9	11,200	50	30	50
Complete fertilizer	4, 12	196 muriate of potash	....	....	100
		340 acid phosphate	....	50	.....
		100 nitrate of soda, 346 dried blood	50	....	.....
Phosphoric acid and potash	6, 10	196 muriate of potash	....	....	100
		340 acid phosphate	....	50	.....
Phosphoric acid	2, 8	340 acid phosphate	....	50	.....

Table I shows the numbers of the plats, the treatment and the amount applied on an acre basis. These amounts were not spread over the entire plat, but were applied to the soil immediately under the trees and extending a little beyond the outer branches so that this area received a very heavy application, especially while the trees were small. The first application was made in the fall of 1899 and spring of 1900 — twenty years ago — but there have been only nineteen seasons of growth which could have been affected by the treatments. The fertilizers have usually been applied between the fifteenth of May and the tenth of June.

Referring again to Figure 34, on the east a lane runs along the side of Plats 1 and 12; on the north is a permanent pasture; on the west, the buffer variety rows extend some distance beyond Plats 6 and 7; on the south from one to two rows of variety trees are planted.

In 1911, the senior author discussed the early history of the trees and the first seven harvests in Bulletin 339, " Is It Necessary to Fertilize an Apple Orchard? " A few quotations from that bulletin will show the conclusions reached at that time.

" The fertilizers have had no sensible effects upon the yield of fruit."

" The size of the apples is possibly increased by the fertilizers."

" A study of the tables giving the diameter of the trees in the experiment shows no difference outside the range of variation in the several plats."

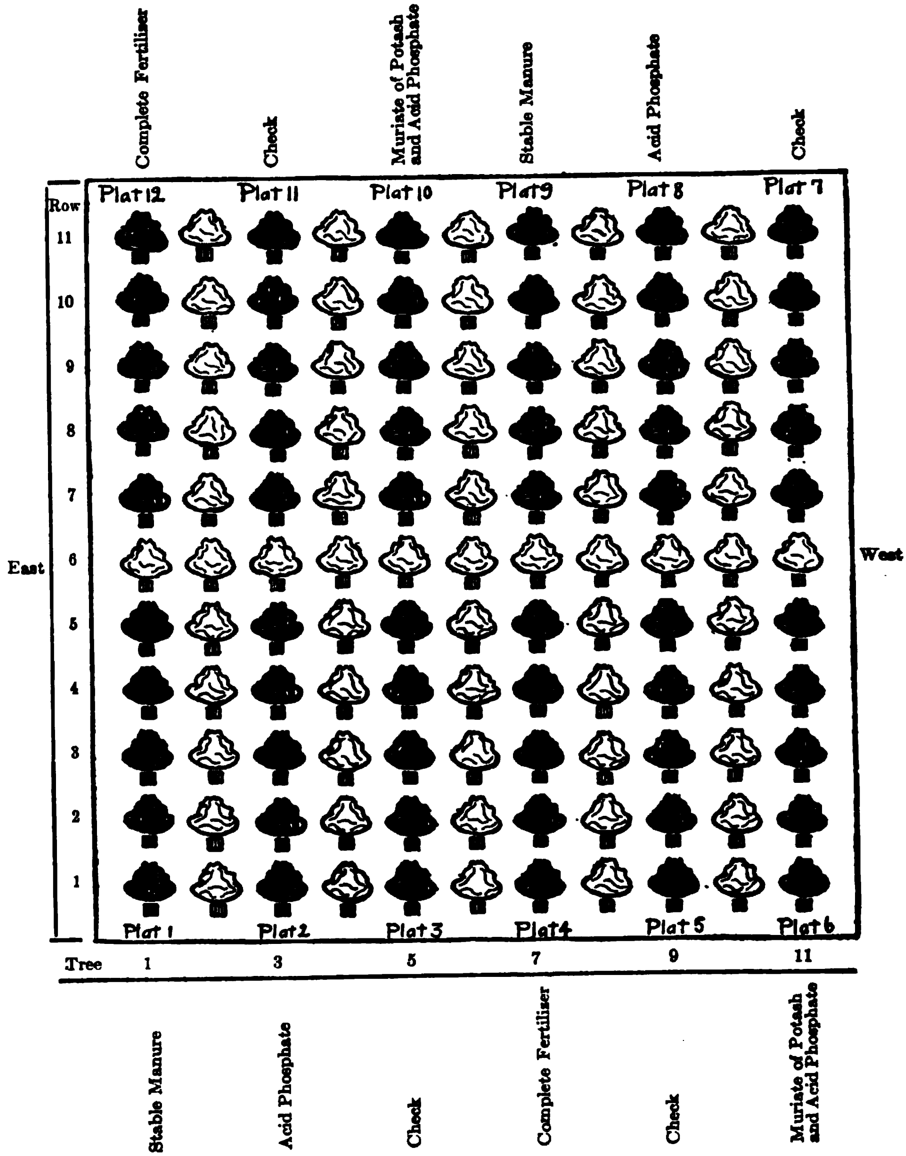

FIG. 34.—PLAN OF ORCHARD AND TREATMENT OF PLATS.

"There was a measurable effect of the nitrogen on the weight of the leaves."

"There is slight evidence that the trees in plats to which nitrogen was applied are making a greater annual growth of branches."

"The trees in this experiment would have been practically as well off had not an ounce of fertilizer been applied."

TREATMENT OF LAND.

In the same year it was planted, the orchard was interplanted with peaches so that the trees stood 20 feet apart. These peaches were left for about nine years. A few times during the early years inter-crops of wheat or rye were harvested, but even then the tree rows were cultivated. In general, the orchard was plowed in the fall or spring and given fair cultivation until the middle of the summer, when a non-leguminous cover-crop was sown. Table II gives the kind of cover-crop and the dates of seeding and plowing since 1910. Similar information for the early years was published in Bulletin 339.

The cultivation has frequently not been sufficient to maintain the soil in the best physical condition, and of late years the growth of cover-crops has not been as heavy as when the orchard was young. It is doubtful if there is as much humus in the soil now as then, or if it could be considered as in as high a state of fertility. At no time during the experiment have there been noticeable differences in the growth of the cover-crop on the various plats.

HARVESTS.

About 200 pounds of fruit were picked in 1902, and seven crops had been harvested when the first report was made, the harvest of 1910 being nearly as large as the six other crops combined. Of the eight additional harvests, several have exceeded the entire yield of the first seven. The crop of 1918 was the largest, with an average of over thirteen bushels per tree.

FACTORS STUDIED.

Reliance in the interpretation of results has been placed chiefly upon two factors: yield of fruit and tree growth. The influence of the various treatments on the size of fruit has also been considered.

TABLE II.— COVER-CROPS IN EXPERIMENTAL ORCHARD.

Year.	Crop.	Sown.	Plowed under.
1911	Barley and cowhorn turnip	7/22	11/23
1912	Buckwheat	7/25	11/9
1913	Barley and oats	7/29	11/18
1914	Rape and cowhorn turnip	8/10	11/28
1915	Barley	8/24	11/12
1916	Rape and cowhorn turnip	8/5	11/4
1917	Rape and cowhorn turnip	8/16	11/12
1918	Rape	8/3	....

INTERPRETATION OF RESULTS.

Critical discussion of orchard.— In every fertilizer experiment there are factors which may influence the results to a greater or less degree and which do not show in the data. Before taking up the results in detail, it may be well to set forth such factors of this kind as are apparent to the authors.

As stated before, previous to planting the orchard the field was used for general farm crops and was considered sufficiently uniform to be desirable for a fertilizer experiment. The history of the orchard, however, shows at least three factors causing lack of uniformity in tree performance.

In the southwest corner of the orchard, Plat 7 lies in a slight depression. The growth of the trees and the yields were so poor that this plat was dropped from the experiment early in its history. As the plat was a check plat, this was especially unfortunate. Beginning with 1917, complete records have again been taken for the plat. This low area also affects Plat 8, but to a much less degree.

In the southeast quarter, there is an area with a much higher proportion of clay in about the middle of Plat 11, another check. Trees on this area have been more productive and, as a result, Plat 11 is quite variable. Probably, this difference in soil also accounts for the high degree of variability in Plat 12. Between this area of high production and Plat 8 there seems to be a gradual decrease in yields due to soil variation.

Just north of the orchard is a small creek valley. A silt loam area along the shoulder of this valley cuts into the orchard as far

as the first trees in Plats 2 and 3. This probably accounts for their decreased yields.

The tree in the fourth row in Plat 5 has a dead area in the lower trunk which has probably decreased its vigor, and prevented normal growth. No reason is known why the fourth tree in Plat 4 should be so low in yield, or why the third in Plat 8 (tree 9, row 9) should be so high in yield.

When the experiment was planned, it was expected that the north and south halves could be used to check against each other. Because of the irregularities discussed above, it does not now seem wise to attempt this, or even to total the two plats receiving the same treatment; instead, the results are usually presented by plats as they stand in the orchard, and the tables might more correctly be called diagrams of the orchard. This enables the reader to make any desired comparison and, at the same time, to judge the effect of the soil variations in the different parts of the orchard.

Probable error of the mean.— In nearly all cases, the result given in the tables is the average, or mean, of the five trees in the plat. With this is given a second value, the probable error of the mean. It is unnecessary to go into the mathematics involved in computing this value,[1] but it is worth while to show its importance in interpreting the results. An example from one of the tables will bring this out. In the table of total yields, Table III, the average yield (the mean value) of the five trees of Plat 11, a check plat, is 4395 pounds with a probable error of ±465 pounds, while the average yield of Plat 5, also a check plat, is 3988 pounds ±148 pounds. In Plat 11, the yield of the individual trees ranged from 2482 pounds to 6039 pounds, a wide range; in Plat 5 the yield ranged from 3858 pounds to 4723 pounds, a narrow range. The value of the probable error depends on the variation of the yield of each tree above or below the average of the five trees, and therefore is large in Plat 11 where there is much variation, and small in Plat 5 where there is little variation. The size of the probable error is, then, an indication of the degree of confidence we may place in the average given

[1] The formula used was:

$$E_m = \pm 0.6745 \sqrt{\frac{\Sigma \ D^2}{n\,(n-1)}}$$

in the tables. In this case it shows us that Plat 5 is a much more reliable check to use in comparisons than is Plat 11.

TABLE III.— RELATION OF POSITION IN ORCHARD TO AVERAGE YIELD PER TREE AND RANK OF PLAT IN ORCHARD FERTILIZER EXPERIMENT.

TOTAL YIELDS IN POUNDS 1902–18.

East.

	Yield.	Rank.		Yield.	Rank.
Plat 1: Manure	4886±202	3	Plat 12: Complete fertilizer	4445±457	7
Plat 2: Phosphorus	4649±230	6	Plat 11: Check	4395±465	8
Plat 3: Check	4742±386	4	Plat 10: Phosphorus and potassium	4728±304	5
North.					*South.*
Plat 4: Complete fertilizer	5202±284	1	Plat 9: Manure	4112±118	9
Plat 5: Check	3988±148	10	Plat 8: Phosphorus	3796±292	11
Plat 6: Phosphorus and potassium	5026±345	2	Plat 7: Check.		

West.

In comparing any two plats with each other, the probable error of their difference is found by squaring the probable sum of each plat, adding these and extracting the square root of the sum. The size of the probable error of the difference in proportion to the difference itself shows how significant such a difference is. Thus in comparing the yield of a fertilized plat with a check plat, if the difference between the two is not as large as the probable error, the difference has no significance; if the difference is twice as large as the probable error, it is suggestive but not conclusive; if three times or larger, we should be justified in assuming that the fertilizer had increased the yield. Of course such a conclusion is reliable only when the soil of the two plats was uniform in the beginning.

In order to avoid confusion, only summary tables are included in the body of the bulletin, but for those who wish to examine the data in more detail, the complete tables are placed in the appendix.

Yield of fruit.— In Table III are given the average yield of the five trees in each plat for the total of fifteen harvests and the relative rank of each plat, Plat 4 with the highest yield being given a rank of one. The best way to analyze this table is to study each treatment by itself.

The phosphorus plats.— There is practically no difference in yield between Plat 2, which has received phosphoric acid, and Plat 3, the adjoining check. Plat 7, the check next to Plat 8 which is the duplicate of Plat 2, is not usable for comparison but, when we compare Plat 8 with Plat 9, and remember that the former is naturally somewhat poorer than the latter, we see that between those two plats there is practically the same relative difference as there is between Plats 2 and 1. From these comparisons we can conclude only that adding acid phosphate at the rate of 340 pounds per acre per year has not given a noticeable increase in yield.

Phosphorus and potassium plats.— There is no reason to believe that Plat 6, which has received acid phosphate and muriate of potash, is naturally more fertile than Plat 5, the adjoining check. In fact, the decrease in yield of Plat 5 as compared with Plat 3 would indicate that the fertility of this quarter probably decreases as we go from east to west. The yield of Plat 6 shows an increase over Plat 5 of more than 1000 pounds per tree in the fifteen harvests, nearly three times the probable error of the difference. When we compare the duplicates, Plats 10 and 11, we see that the difference is less than the probable error, but we must remember that Plat 10 is very probably somewhat lower in natural fertility than Plat 11. This would seem to justify us in concluding that adding 196 pounds of muriate of potash each year to the 340 pounds of acid phosphate has resulted in an increased yield. Before discussing this further let us take up the other two treatments.

Complete fertilizer plats.— In studying Plat 4, it seems best to use the average of the two checks which lie on both sides of it. Using this average, we find there has been an increased yield of over 1000 pounds per tree, over two times the probable error of the difference. However, when we compare the duplicate plats, we see that the yield of Plat 12 is not appreciably better than Plat 11, the adjoining check, and apparently not quite as good as Plat 10, though we have reason to believe that Plat 10 is not as favorably located as Plat 12. Also, when we compare the complete fertilizer plat, 4, with Plat 6 which receives the same amount of phosphoric acid and potash but no nitrogen, there is no significant difference. It would seem that annual applications of 50 pounds per acre of readily available nitrogen in addition to the phosphoric acid and potash have caused no increase in yields.

Manure plats.— Manure has been added annually at a rate sufficient to supply 50 pounds of nitrogen per acre. This has also carried about 30 pounds of phosphoric acid and about 50 pounds of potash. Plat 1 is no better in yield than Plat 3, the check, nor significantly better than Plat 2, and, when we consider the probable relative fertility, Plat 9, the duplicate of Plat 1, is no better than Plat 8, the duplicate of Plat 2.

General conclusions from yields.— The phosphoric acid has been of no benefit when used alone. Potash combined with the phosphoric acid would seem to have increased yields, but the results with the same materials in a complete fertilizer and over one-half the amount in a less available form in manure are so conflicting that we can place but little reliance on such a conclusion.

The addition of nitrogen to the phosphoric acid and potash and the same amount of nitrogen in the manure has caused no apparent increase in yield.

In general, there are so many inconclusive or contradictory results that no conclusion of practical value can be drawn from the yields.

Before leaving the discussion of yields, it is well to call attention to Table IV, which shows the relative rank in yield of the various plats at the time of the first report and for each year since. It

TABLE IV.— RANK OF PLATS IN ORCHARD FERTILIZER EXPERIMENT YIELDS.

Plat Treatment.	1902–1910.	1911.	1912.*	1913.	1914.	1915.	1916.	1917.	1918.	1911–1918.
1. Manure.......	3	2	2	1	3	10	6	11	3	3
9. Manure.......	10	8	8	9	10	3	7	9	8	9
2. Phosphorus....	4	5	3	3	5	8	9	8	4	6
8. Phosphorus....	11	11	7	7	11	9	11	6	9	11
6. Phosphorus and potassium...	2	4	10	4	1	4	1	7	1	2
10. Phosphorus and potassium...	6	7	6	11	7	2	2	1	2	4
4. Complete fertilizer.......	1	1	9	2	2	1	3	2	5	1
12. Complete fertilizer.......	8	10	1	5	8	6	5	3	11	7
3. Check........	5	3	4	6	4	5	8	4	6	5
5. Check........	9	9	11	8	6	11	10	10	10	10
11. Check..	7	6	5	10	9	7	4	5	7	8
7. Check........	....	...	....	...	...	...	...	...	...	.....

* Light crop.

would be expected that, if a fertilizer were influencing the final results, it would show a cumulative effect as we study the plat from year to year. When we compare the rank for the period ending in 1910 with the rank for the last eight harvests, we see that two of the checks have dropped back one place each in rank, and one of the plats receiving phosphoric acid has dropped two places, while one of the plats receiving phosphoric acid and potash has come up two places, and one of the complete fertilizer plats one place. If this shift continues, it will be very significant, but at present it serves simply to show how little eight years of fertilization has changed the results, since the differences between the various ranks are so small; for example, the difference for the last eight harvests between Plat 10, with a rank of four, and Plat 3, with a rank of five, is only 14 pounds per tree.

Size of fruit.— Table V gives the percentage of the total yield of each plat that was graded as two and one-half inches or over. When

TABLE V.— INFLUENCE OF TREATMENT ON SIZE OF FRUIT. PERCENTAGE OF TOTAL YIELD OF PLAT MEASURING TWO AND ONE-HALF INCHES OR LARGER.

	Per ct.	*East.*	*Per ct.*
Plat 1: Manure	84.5±1.68	Plat 12: Complete fertilizer	81.5±1.14
Plat 2: Phosphorus	80.8± .76	Plat 11: Check	79.7±1.16
Plat 3: Check	78.6±1.5	Plat 10: Phosphorus and potassium	83.9±1.06
North.			*South.*
Plat 4: Complete fertilizer	86.7± .89	Plat 9: Manure	83.9± .79
Plat 5: Check	86.8± .71	Plat 8: Phosphorus	84.4± .71
Plat 6: Phosphorus and potassium	89.2± .52	Plat 7: Check	....
		West.	

we examine this table, we see immediately that there is a greater difference between Plats 3 and 5, both checks, than between any fertilizer plat and its nearest check. At first thought we might surmise that the heavier yield of Plat 3 had resulted in smaller fruits, but when we study all the plats we find that, if there is any correlation between yield and size, it is probably in the direction of larger fruit with larger yields. This unaccountable difference between two check plats makes any explanation of the differences

among the fertilized plats uncertain, and a study of a few of the plats increases this uncertainty. Thus, Plat 1 has a larger proportion of large fruit than Plat 2, but this is not the case with their duplicates. Plat 4, complete fertilizer, is the same as Plat 5, a check. Plat 6 has the largest proportion of large fruit in the test, but its duplicate is the same as Plat 9, and the same or slightly lower than Plat 8.

It should be borne in mind that Rome is a variety which seldom overloads and so, even with large yields, each apple gets a chance to reach normal size. With other varieties we should probably find a marked tendency for the size of fruit to decrease with large yields.

As in the case of yield, the results are too conflicting for us to draw any certain conclusion.

Tree growth.— Table VI gives the average trunk diameter and the approximate average tree volume for each plat. As all the

TABLE VI.— TRUNK DIAMETERS AND APPROXIMATE TREE VOLUMES. AVERAGE PER TREE PER PLAT.

East.

	Trunk diameter, inches.	Tree volume, cu. ft.		Trunk diameter, inches.	Tree volume, cu. ft.
Plat 1: Manure	9.5±.11	1491±56	Plat 12: Complete fertiliser	9.0±.22	1320±80
Plat 2: Phosphorus	9.6±.14	1357±54	Plat 11: Check	9.2±.23	1321±85
Plat 3: Check	9.5±.10	1535±64	Plat 10: Phosphorus and potassium	9.7±.21	1555±91
North.					*South.*
Plat 4: Complete fertiliser	9.8±.23	1624±91	Plat 9: Manure	9.4±.12	1255±34
Plat 5: Check	9.0±.21	1291±98	Plat 8: Phosphorus	8.7±.14	1249±41
Plat 6: Phosphorus and potassium	10.2±.11	1601±92	Plat 7: Check	8.1±.24	968±57

West.

trees were the same diameter when planted, the trunk diameters in 1918 show the relative increases. These diameters were obtained by measuring the trunks one foot and three feet from the ground, and averaging the two.

The values used for tree volumes do not give the exact space occupied by the tops, but they are near enough to the true values so that they may be safely used for comparison.[1]

These two factors are excellent ones to use in comparing the various plats, because they are not seriously influenced by minor seasonal fluctuations which might have a very considerable effect upon the fruit.

The results from the four checks would seem to show, as well as we can judge, the relative fertility of the four locations, tho Plat 11 is less variable than we would expect from its yield record.

As we have no reason to assume that phosphorus has been injurious, the decreased tree volume in Plat 2 is probably due to soil or tree variations, tho this volume is unusually small in proportion to the trunk diameter. This does not seem to hold for Plat 8, the second phosphorus plat.

The phosphorus and potassium plats lead their adjoining plats both in size of trunk and tree volume. In this respect they are relatively as good or better than they were in yield of fruit. But when we add nitrogen to these two elements in Plat 12, complete fertilizer, the tree trunks and volumes are no larger than the adjacent check; and in Plat 4, tho both values are better than the average of the two adjacent checks, the trunk diameter is less than with the two elements only and the tree volume is not significantly larger. With these variations in the complete fertilizer plats, it is not possible to say whether the increases in Plats 6 and 10 are due to the potassium or to the combination of the potassium with the phosphorus or to some tree or field variation which does not now show.

[1] This volume was obtained in the following way: The diameter of the tree was measured in an east and west direction, and the height from the lowest branches to the top was found. The volume of the cone with a base equal to the spread of the tree and a height equal to the tree height would be:

$$\frac{1}{3} \times \frac{22}{7} \times \frac{(\text{diameter})^2}{2} \times \text{height or}$$

$$\frac{22}{21} \times \frac{(\text{diameter})^2}{2} \times \text{height}$$

As $\frac{22}{21}$ was common to all the volumes and nearly equal to unity, it was dropped, and the figures in Table VI represent $\frac{(\text{diameter})^2}{2} \times \text{height}$. The Rome trees carry a central leader with relatively small side branches, and are more nearly conical than most sorts, so the above computation is close to the true space occupied by the tops.

One of the most surprising results is the failure of the manure treatment in both Plat 1 and Plat 9 to show any significant increase in tree growth. Certainly, with this the case and with the complete fertilizer plats poorer than those receiving only phosphorus and potassium one can not say that heavy additions of nitrogen have caused excessive growth.

In the case of tree growth as with yields, tho certain treatments have resulted in increases, the results are too uncertain to lead to any definite conclusion.

Costs.— The final question in all fertilizer experiments is, of course, whether any increased yields secured are sufficient to pay the costs involved. The total cost per tree of the various treatments, excluding the cost of mixing and applying has been roughly as follows: phosphoric acid, $1.75; phosphoric acid and potash, $5.25; complete fertilizer, $13; stable manure, $11.75. If we refer back to the discussion of yield of fruit, we will see that certain plats have given increases sufficient to pay these and the other costs involved in the use of fertilizers, or even to show a profit, as in Plat 6, which seems to show an increase of over 1000 pounds per tree at a cost of $5.25; but in other plats the same plant food elements have shown a financial loss.

GENERAL CONCLUSIONS.

The combination of phosphoric acid and potash and the complete fertilizer treatment have caused small increases in yield and probably somewhat more vigorous tree growth. Manure did not cause an appreciable increase in yield or growth, nor was the addition of nitrogen in the complete fertilizer of measurable value in growth. Phosphoric acid used alone has been of no value.

If the results continue in the present direction for another ten years, the increased yields may justify the recommendation of one or two of these treatments, but at present they are too irregular and the increases too small to show any certain financial benefit.

In applying these conclusions in practical orcharding, it should be borne in mind that they are the result of work in a cultivated orchard on soil naturally well supplied with the plant food elements. On thin, infertile soils, or in sod orchards, the results might be quite different.

APPENDIX.

TABLE I.— YEARLY YIELDS OF INDIVIDUAL TREES IN AN ORCHARD FERTILIZER EXPERIMENT.

	Location.		1911.	1912.	1913.	1914.	1915.	1916.	1917.	1918.	Total, 1902–1910.	Grand total.	Departures from mean.
	Row No.	Tree No.											
			Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*		
Plat 1. Stable manure.	1	1	754	198	604	590	641	433	401	613	700	4,934	+48
	2	1	673	298	542	642	559	282	541	604	855	4,996	+110
	3	1	766	369	466	778	554	503	342	936	899	5,613	+727
	4	1	524	219	227	598	447	444	249	693	385	3,786	—1,100
	5	1	608	263	379	565	724	618	296	745	902	5,100	+214
Total	.....	.....	3,325	1,347	2,218	3,173	2,925	2,280	1,829	3,591	3,741	24,429	....
Average per tree	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	4,886	....
Plat 9. Stable manure.	7	7	698	177	274	448	812	482	556	635	651	4,733	+621
	8	7	624	145	234	409	702	301	465	606	604	4,090	—22
	9	7	507	186	251	442	768	470	312	703	523	4,162	+50
	10	7	503	182	196	486	655	447	296	706	394	3,865	—247
	11	7	509	87	254	466	695	549	258	470	420	3,708	—404
Total	.....	.....	2,841	777	1,209	2,251	3,632	2,249	1,887	3,119	2,592	20,558	....
Average per tree	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	4,112	....
Treatment total	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	6,333	44,987	....

Plat 2. Phosphoric acid.	1	3	473	102	372	430	403	332	304	507	517	3,440	—1,209
	2	3	651	268	523	536	634	241	446	783	999	5.081	+432
	3	3	721	437	330	739	599	485	306	918	832	5,367	+718
	4	3	*631	243	412	558	608	378	380	698	478	4,386	—263
	5	3	679	163	423	527	795	458	464	585	877	4.971	+322
Total.........	.....	.....	3,155	1,213	2,060	2,790	3,039	1,894	1,900	3,491	3,703	23,245	....
Average per tree...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	4,649	....
Plat 8. Phosphoric acid.	7	9	606	247	289	547	613	350	400	669	526	4,247	+451
	8	9	358	138	127	454	481	233	278	565	246	2,880	—916
	9	9	642	292	460	491	749	399	552	895	783	5,263	+1,467
	10	9	464	63	283	212	576	309	393	513	333	3,146	—650
	11	9	484	219	336	258	581	283	357	388	539	3,445	—351
Total.........	.....	.....	2,554	959	1,495	1,962	3,000	1,574	1,980	3,030	2,427	18,981	....
Average per tree...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3,796	....
Treatment total....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	6,131	42,226	....

* Tree injured and its record discarded and averages of remaining 4 trees used in computations.

TABLE I.—YEARLY YIELDS OF INDIVIDUAL TREES IN AN ORCHARD FERTILIZER EXPERIMENT (*continued*).

	Location.		1911.	1912.	1913.	1914.	1915.	1916.	1917.	1918.	Total, 1902–1910.	Grand total.	Departures from mean.
	Row No.	Tree No.											
			Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*		
Plat 6. Phosphoric acid and potash.	1	11	725	90	479	801	771	672	237	749	943	5,467	+441
	2	11	590	51	451	637	676	489	334	693	713	4,634	—392
	3	11	562	43	275	601	598	476	334	742	483	4,114	—912
	4	11	831	336	516	832	877	788	694	914	1,023	6,811	+1,785
	5	11	496	183	275	608	626	422	346	555	593	4,104	—922
Total.........	.....	.....	3,204	703	1,996	3,479	3,548	2,847	1,945	3,653	3,755	25,130	....
Average per tree...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	5,026	....
Plat 10. Phosphoric acid and potash.	7	5	736	266	299	702	889	454	799	764	887	5,796	+1,068
	8	5	597	26	119	484	761	485	406	756	568	4,202	—526
	9	5	644	114	219	590	980	681	521	867	715	5,331	+603
	10	5	435	448	320	556	609	576	534	743	832	5,053	+325
	11	5	432	107	133	303	469	340	502	493	477	3,256	—1,472
Total.......	.....	.....	2,844	961	1,090	2,635	3,708	2,536	2,762	3,623	3,479	23,638	....
Average per tree...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	4,728	....
Treatment total....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	7,235	48,768	....

Plat 4. Phosphoric acid and potash and nitrogen.	1	7	764	271	631	955	888	642	439	896	1,053	6,539	+1,137
	2	7	759	102	590	676	816	478	645	656	943	5,665	+463
	3	7	878	207	388	766	777	614	331	831	703	5,495	+293
	4	7	351	48	68	389	428	403	330	509	304	2,830	—1,372
	5	7	738	140	420	573	1,012	393	778	542	886	5,482	+280
Total	.....	.....	3,490	768	2,097	3,359	3,921	2,530	2,523	3,434	3,889	26,011	....
Average per tree	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	5,202	....
Plat 12. Phosphoric acid and potash and nitrogen.	7	1	592	245	347	623	723	475	448	485	430	4,368	—77
	8	1	379	141	177	339	440	296	321	343	275	2,711	—1,734
	9	1	675	174	601	213	920	490	649	803	968	5,493	+1,048
	10	1	705	582	422	707	825	660	644	778	1,055	6,378	+1,933
	11	1	413	223	156	585	412	458	237	370	420	3,274	—1,171
Total	.....	.....	2,764	1,365	1,703	2,467	3,320	2,379	2,299	2,779	3,148	22,224	....
Average per tree	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	4,445	....
Treatment total	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	7,038	48,235	....

TABLE I.— YEARLY YIELDS OF INDIVIDUAL TREES IN AN ORCHARD FERTILIZER EXPERIMENT (*concluded*).

	Location.		1911.	1912.	1913.	1914.	1915.	1916.	1917.	1918.	Total, 1902–1910.	Grand total.	Departures from mean.
	Row No.	Tree No.											
			Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*		
Plat 3. Check.	1	5	411	65	184	427	413	286	153	433	312	2,684	—2,058
	2	5	601	274	411	621	644	377	390	745	663	4,726	—16
	3	5	712	250	346	586	620	408	524	765	741	4,952	+210
	4	5	686	277	285	707	772	435	614	678	728	5,182	+440
	5	5	816	259	358	705	994	588	588	737	1,121	6,166	+1,424
Total.........	.....	.....	3,226	1,125	1,584	3,046	3,443	2,094	2,269	3,358	3,565	23,710	....
Average per tree...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	4,742	....
Plat 5. Check.	1	9	608	87	348	569	527	282	319	546	572	3,858	—127
	2	9	497	129	321	558	562	288	382	615	661	4,013	+28
	3	9	602	186	311	668	759	370	418	766	643	4,723	+738
	4	9	480	69	218	448	438	345	399	533	415	3,345	—640
	5	9	600	174	204	545	581	458	366	519	555	4,002	+17
Total.........	.....	.....	2,787	645	1,402	2,788	2,867	1,743	1,884	2,979	2,846	19,941	....
Average per tree...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3,988	....

Plat 11. Check.	7	3	388	27	105	304	437	230	288	435	259	2,482	—1,914
	8	3	453	56	174	341	428	319	344	470	525	3,110	—1,286
	9	3	746	163	362	572	892	547	537	848	867	5,534	+1,138
	10	3	709	370	335	624	940	686	659	776	940	6,039	+1,643
	11	3	644	413	207	538	503	617	434	773	684	4,813	+417
Total.........	.....	.....	2,940	1,029	1,183	2,379	3,200	2,408	2,262	3,302	3,275	21,978	....
Average per tree...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	4,395	....
Plat 7. Check.	7	11	.....	.....	.....	.....	.....	.....	172	375	.....	......	....
	8	11	.....	.....	.....	.....	.....	.....	19	207	.....	......	....
	9	11	.....	.....	.....	.....	.....	.....	35	387	.....	......	....
	10	11	.....	.....	.....	.....	.....	.....	35	398	.....	......	....
	11	11	.....	.....	.....	.....	.....	.....	261	723	.....	......	....
Total.........	.....	.....	.....	.....	.....	.....	.....	.....	522	2,090	.....	......	....

TABLE II.— INFLUENCE OF TREATMENT ON SIZE OF FRUIT IN ORCHARD FERTILIZER EXPERIMENT.

POUNDS OF FRUIT GRADING TWO AND ONE-HALF INCHES OR LARGER.

	Location. Row No.	Location. Tree No.	1911.	1912.	1913.	1914.	1915.	1916.	1917.	1918.	Total.	Total yield.	Percentage 2½ inches or larger.	Departures from mean.
Plat 1. Stable manure.	1	1	641	187	539	487	612	405	377	566	3,814	4,236	90.0	+ 5.5
	2	1	547	278	494	454	511	264	486	560	3,594	4,144	86.7	+ 2.2
	3	1	652	349	399	564	502	461	318	859	4,104	4,720	86.9	+ 2.4
	4	1	375	199	187	383	424	158	226	557	2,509	3,407	73.6	—10.9
	5	1	440	240	338	412	669	418	287	674	3,478	4,200	82.8	— 1.7
Total...........	......	......	2,655	1,253	1,957	2,300	2,718	1,706	1,694	3,216	17,499	20,707	84.5	....
Plat 9. Stable manure.	7	7	518	161	195	377	706	423	437	595	3,412	4,088	83.4	— .5
	8	7	520	135	155	355	639	279	380	566	3,029	3,495	86.6	+2.7
	9	7	381	171	197	359	661	402	297	615	3,083	3,644	84.6	+ .7
	10	7	412	169	148	416	601	340	264	609	2,959	3,477	85.1	+1.2
	11	7	408	83	209	399	656	379	226	266	2,626	3,295	79.6	—4.3
Total...........	......	......	2,239	719	904	1,906	3,263	1,823	1,604	2,651	15,109	17,999	83.9	....
Plat 2. Phosphoric acid.	1	3	310	79	329	296	385	306	277	444	2,426	2,932	82.7	+1.9
	2	3	442	224	434	292	584	218	408	671	3,273	4,085	80.1	— .7
	3	3	575	388	284	396	548	464	279	846	3,779	4,536	83.3	+2.5
	*4	3	437	210	344	333	536	345	347	616	3,168	3,925	80.8	.0
	5	3	420	151	330	348	626	391	424	503	3,193	4,106	77.7	—3.1
Total...........	......	......	2,184	1,052	1,721	1,665	2,679	1,724	1,735	3,080	15,839	19,584	80.8	....
Plat 8. Phosphoric acid.	7	9	477	207	219	429	554	332	352	624	3,194	3,730	85.6	+1.2
	8	9	284	118	80	341	433	210	231	501	2,198	2,650	82.9	—1.5
	9	9	521	270	267	437	697	371	521	771	3,855	4,483	84.9	+ .5
	10	9	375	58	167	194	501	290	349	490	2,424	2,818	86.01	+1.6
	11	9	385	179	195	214	510	244	274	343	2,344	2,921	80.2	—4.0
Total...........	......	......	2,042	832	928	1,615	2,695	1,447	1,727	2,729	14,015	16,602	84.4	....

Plat 6. Phosphoric acid and potash.	1	11	557	79	442	641	713	609	229	636	3,906	4,457	87.6	—1.6
	2	11	454	46	424	553	635	471	324	660	3,567	3,930	90.7	+1.5
	3	11	458	39	255	497	564	464	325	727	3,329	3,638	91.5	+2.3
	4	11	654	291	463	636	823	742	646	874	5,129	5,792	88.5	— .7
	5	11	417	151	245	479	567	383	337	518	3,097	3,517	88.0	—1.2
Total	......	......	2,540	606	1,829	2,806	3,302	2,669	1,861	3,415	19,028	21,334	89.2	....
Plat 10 Phosphoric acid and potash.	7	5	582	220	222	493	791	437	601	706	4,052	4,915	82.4	—1.5
	8	5	406	22	79	360	686	385	371	653	2,962	3,642	81.3	—2.6
	9	5	478	107	168	432	862	610	469	784	3,910	4,625	84.5	+ .6
	10	5	374	411	272	399	569	539	500	715	3,779	4,227	89.4	+5.5
	11	5	261	97	72	248	438	252	396	435	2,199	2,724	80.7	—3.2
Total	......	......	2,101	857	813	2,130	3,346	2,223	2,337	3,293	16,902	20,133	83.9	....
Plat 4. Complete fertilizer.	1	7	636	234	576	703	760	602	413	833	4,757	5,493	86.6	— .1
	2	7	670	89	521	555	741	454	582	624	4,236	4,727	89.6	+2.9
	3	7	781	187	357	502	751	581	316	797	4,272	4,797	89.0	+2.3
	4	7	235	42	53	260	411	361	303	470	2,135	2,534	84.2	—2.5
	5	7	577	128	326	513	716	375	691	506	3,832	4,627	82.8	—3.9
Total	......	......	2,899	680	1,833	2,532	3,379	2,373	2,305	3,230	19,232	22,178	86.7	....
Plat 12. Complete fertilizer.	7	1	500	228	278	445	685	363	375	466	3,340	3,944	84.6	+3.1
	8	1	288	124	146	197	423	226	274	325	2,003	2,442	82.0	+ .5
	9	1	462	163	352	129	770	447	508	750	3,581	4,537	78.9	—2.6
	10	1	600	514	346	418	748	514	597	755	4,492	5,326	84.3	+2.8
	11	1	329	209	238	208	394	234	225	339	2,176	2,873	75.7	—5.8
Total	......	......	2,179	1,238	1,360	1,397	3,020	1,784	1,979	2,635	15,592	19,122	81.5	....
Plat 3. Check.	1	5	203	52	143	182	377	265	140	321	1,683	2,374	70.8	—7.8
	2	5	431	250	356	350	562	367	359	634	3,309	4,071	81.2	+2.6
	3	5	361	161	243	337	524	391	465	703	3,185	4,218	75.5	—3.1
	4	5	480	219	227	324	616	417	531	629	3,443	4,459	77.2	—1.4
	5	5	584	233	262	541	823	550	554	700	4,247	5,052	84.	+5.4
Total	......	......	2,059	915	1,231	1,734	2,902	1,990	2,049	2,987	15,867	20,174	78.6	....

* Tree injured and record discarded. Used average of remaining 4 in computations.

TABLE II.— INFLUENCE OF TREATMENT ON SIZE OF FRUIT IN ORCHARD FERTILIZER EXPERIMENT (*concluded*).

POUNDS OF FRUIT GRADING TWO AND ONE-HALF INCHES OR LARGER.

	Location.		1911.	1912.	1913.	1914.	1915.	1916.	1917.	1918.	Total	Total yield.	Percentage 2½ inches or larger.	Departures from mean.
	Row No.	Tree No.												
Plat 5. Check.	1	9	384	79	300	513	469	274	293	443	2,755	3,289	83.7	—3.1
	2	9	321	117	299	441	516	280	359	567	2,900	3,361	86.2	— .4
	3	9	472	161	282	574	703	360	405	738	3,695	4,084	90.4	+3.6
	4	9	317	62	173	354	416	338	378	489	2,527	2,928	86.3	— .5
	5	9	518	153	164	442	521	420	355	450	3,023	3,502	86.3	— .5
Total	......	......	2,012	572	1,218	2,324	2,625	1,672	1,790	2,687	14,900	17,164	86.8	....
Plat 11. Check.	7	3	206	21	64	185	378	208	249	373	1,684	2,231	75.4	—4.3
	8	3	319	50	96	183	404	257	285	421	2,015	2,594	77.6	—2.1
	9	3	530	149	248	475	803	475	489	759	3,928	4,676	83.9	+4.2
	10	3	567	320	259	337	905	482	584	730	4,184	5,103	81.9	+2.2
	11	3	455	355	143	203	477	433	397	677	3,140	4,135	75.9	—3.8
Total	......	......	2,077	895	810	1,383	2,967	1,855	2,004	2,960	14,951	18,739	79.7	....
Plat 7. Check.	7	......	......	......	......	......	......	......	167	362	......	......	......	......
	8	......	......	......	......	......	......	......	18	174	......	......	......	......
	9	......	......	......	......	......	......	......	33	340	......	......	......	......
	10	......	......	......	......	......	......	......	29	338	......	......	......	......
	11	......	......	......	......	......	......	......	227	655	......	......	......	......
Total	......	......	......	......	......	......	......	......	474	1,869	......	......	......	......

Table III.— Approximate Tree Volumes in Orchard Fertilizer Experiment.

		Row.	Tree.	Height. Feet.	Spread. Feet.	$\left(\frac{\text{Spread}}{2}\right)$	$\left(\frac{\text{Spread}}{2}\right)^2$	$\left(\frac{\text{Spread}}{2}\right)^2$ x Height
Stable manure.	Plat 1.	1	1	13.5	21.0	10.5	110.2	1,487.7
		2	1	12.5	21.0	10.5	110.2	1,377.5
		3	1	13.5	22.5	11.25	126.5	1,707.75
		4	1	12.5	20.0	10.0	100.0	1,250.0
		5	1	13.5	22.0	11.0	121.0	1,633.5
Total.	......	...	...	......	......	....	....	7,456.45
Average..	......	...	...	......	......	....	....	1,491.29
	Plat 9.	7	7	12.0	21.0	10.5	110.2	1,322.4
		8	7	11.5	19.5	9.75	95.0	1,092.5
		9	7	12.0	20.0	10.0	100.0	1,200.0
		10	7	11.5	22.0	11.0	121.0	1,391.5
		11	7	11.0	21.5	10.75	115.5	1,270.5
Total.	......	...	...	......	......	....	....	6,276.9
Average..	......	...	...	......	......	....	....	1,255.3
Treatment total...	......	...	...	......	......	....	....	13,733.35
Phosphoric acid.	Plat 2.	1	3	12.0	19.5	9.75	95.0	1,140.0
		2	3	11.5	22.0	11.0	121.0	1,391.5
		3	3	13.0	22.0	11.0	121.0	1,573.0
		4	3	......	......	....	....	*1,356.7
		5	3	12.0	21.0	10.5	110.2	1,322.4
Total.	......	...	...	......	......	....	....	6,783.6
Average..	......	...	...	......	......	....	....	1,356.7
	Plat 8.	7	9	11.5	21.5	10.75	115.5	1,328.25
		8	9	10.5	20.5	10.25	105.0	1,102.5
		9	9	11.5	22.0	11.00	121.0	1,391.5
		10	9	12.0	21.0	10.50	110.2	1,322.4
		11	9	11.0	20.0	10.00	100.0	1,100.0
Total.	......	...	...	......	......	....	....	6,244.65
Average..	......	...	...	......	......	....	....	1,248.93
Treatment total...	......	...	...	......	......	....	....	13,028.25
Phosphoric acid and potash.	Plat 6.	1	11	14.0	23.0	11.5	132.2	1,850.8
		2	11	14.0	21.0	10.5	110.2	1,542.8
		3	11	14.0	21.0	10.5	110.2	1,542.8
		4	11	14.5	23.0	11.5	132.2	1,916.9
		5	11	11.5	20.0	10.0	100.0	1,150.0
Total.	......	...	...	......	......	....	....	8,003.3
Average..	......	...	...	......	......	....	....	1,600.6
	Plat 10.	7	5	13.0	23.0	11.5	132.2	1,718.6
		8	5	13.5	23.0	11.5	132.2	1,784.7
		9	5	13.5	23.0	11.5	132.2	1,784.7
		10	5	12.0	21.5	10.75	115.5	1,386.0
		11	5	11.0	20.0	10.0	100.0	1,100.0
Total.	......	...	...	......	......	....	....	7,774.0
Average..	......	...	...	......	......	....	....	1,555.0
Treatment total...	......	...	...	......	......	....	....	15,777.3

* Trunk injured; used average of four.

TABLE III (*concluded*).

		Row.	Tree.	Height. Feet.	Spread. Feet	$\left(\frac{\text{Spread}}{2}\right)$	$\left(\frac{\text{Spread}}{2}\right)^2$	$\left(\frac{\text{Spread}}{2}\right)^2$ x Height
Complete fertilizer.	Plat 4.	1	7	13.0	23.5	11.75	138.0	1,694.0
		2	7	12.5	23.5	11.75	138.0	1,725.0
		3	7	12.5	24.5	12.25	150.0	1,875.0
		4	7	11.0	20.0	10.00	100.0	1,100.0
		5	7	12.5	23.5	11.75	138.0	1,725.0
Total.	......	...	...	......	......	....	....	8,119.0
Average..	......	...	...	......	......	....	....	1.624.0
	Plat 12.	7	1	12.5	21.5	10.75	115.5	1,443.75
		8	1	11.5	19.0	9.50	90.2	1,037.3
		9	1	12.0	20.0	10.00	100.0	1,200.0
		10	1	13.0	23.0	11.50	132.2	1,718.6
		11	1	12.0	20.0	10.00	100.0	1,200.0
Total.	......	...	...	......	......	....	....	6,599.65
Average..	......	...	...	......	......	....	....	1,319.93
Treatment total...	......	...	...	......	......	....	....	14,718.65
Check.	Plat 3.	1	5	11.5	20.5	10.25	105.0	1,207.5
		2	5	12.5	21.5	10.75	115.5	1,443.75
		3	5	13.5	22.0	11.00	121.0	1,633.5
		4	5	14.5	22.0	11.00	121.0	1,754.5
		5	5	13.5	22.0	11.00	121.0	1,633.5
Total.	......	...	...	......	......	....	....	7,672.75
Average..	......	...	...	......	......	....	....	1,534.55
	Plat 5.	1	9	11.5	20.5	10.25	105.0	1,207.5
		2	9	11.0	19.5	9.75	95.0	1,045.0
		3	9	14.0	22.5	11.25	126.5	1,771.0
		4	9	10.0	20.0	10.00	100.0	1,000.0
		5	9	13.0	21.0	10.50	110.2	1,430.0
Total.	......	...	...	......	......	....	....	6,453.5
Average..	......	...	...	......	......	....	....	1,290.7
	Plat 11.	7	3	10.5	18.5	9.25	85.5	897.75
		8	3	11.5	20.5	10.25	105.0	1,207.5
		9	3	13.0	21.0	10.50	110.2	1,430.0
		10	3	12.5	23.0	11.50	132.2	1,652.5
		11	3	13.5	20.5	10.25	105.0	1,417.5
Total.	......	...	...	......	......	....	....	6,605.25
Average..	......	...	...	......	......	....	....	1,321.05
Treatment total...	......	...	...	......	......	....	....	20,731.5
	Plat 7.	7	11	10.0	20.5	10.25	105.0	1,050.0
		8	11	8.5	18.0	9.0	81.0	688.5
		9	11	10.0	20.0	10.0	100.0	1,000.0
		10	11	10.0	19.0	9.5	90.2	900.0
		11	11	12.0	20.0	10.0	100.0	1,200.0
Total.	......	...	...	......	......	....	....	4,838.5
Average..	......	...	...	......	......	....	....	967.7

A TEST OF METHODS IN PRUNING THE CONCORD GRAPE IN THE CHAUTAUQUA GRAPE BELT*

F. E. GLADWIN.

SUMMARY.

The methods of training the vine common to eastern United States may be placed in three groups according to the position the fruiting shoots assume, namely, the drooping, the upright, and the horizontal types.

Tests conducted at Fredonia during the past eight years with seven methods representing the drooping and the upright types indicate that increased yields cannot be expected by merely changing the method of training.

The Single-stem Kniffin, the Umbrella Kniffin, the Munson and the Chautauqua methods are about equally useful, so far as yield is concerned, for training the Concord. Under conditions prevailing at Fredonia, the High Renewal and the Two-stem Kniffin have produced less and inferior fruit.

From a consideration of all the advantages and disadvantages of the various methods and from the data covering the yields, wood production and quality of the fruit, it is found that the Single-stem Kniffin outranks any other method for training the Concord in the Chautauqua Belt.

The tests indicate that the period of pruning, early winter compared with spring pruning, has not exerted sufficient influence upon yield, wood growth nor upon the maturity of the fruit to warrant stating a preference.

Spring-pruning, however, is preferable to early winter pruning, for the reason that a better selection of fruit canes may be made after the severe temperatures of winter have passed, than can be done earlier.

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INTRODUCTION.

The grape grower's art is largely carried on by pruning tools. No other orchard plant is so greatly transformed from its natural habit of growth as the grape. Happily, it thrives " under the knife," and the vine may be trained and pruned in diverse ways without injury to the plant. It follows that there are many methods of pruning grapes, a dozen or more being used in the commercial vineyards of eastern America. Pruning as a rule, however, follows accepted patterns in the various grape regions of the country, and there seem to have been very few experiments to determine whether the methods adopted are the best.

This bulletin is the account of an experiment to determine which of the various methods commonly practiced in pruning are best for the Concord grape in the great Concord region known as the Chautauqua Grape Belt. The experiment was started in 1911 in a vineyard maintained by this Station at Fredonia, New York, in the heart of the Chautauqua region. The data given are for the first eight years of the experiment.

SITE, SOIL AND CONDITIONS OF THE EXPERIMENT.

The vineyard in which the experiment was carried on was planted in 1907 on a clay loam soil underlaid by shale. It covered about one and one-quarter acres. The vines were planted in rows eight feet apart, and six feet apart in the rows. The plats discussed in this report consist of two rows of seventy-two vines each. It must be noted that there are 907 vines to the acre in this experiment as compared with 680 vines to the acre as the Concord is commonly planted in the Chautauqua region. The vines bore a small crop in 1909 up until which time they had been pruned to the Chautauqua method generally used in this region. In the winter of 1910 each plat was pruned as closely as possible to conform with the method that it was proposed to test. In the pruning of 1911 all the vines had been remodeled. Of the score or more methods that might have been tested but seven were selected for this experiment. Those culled out were such as were too complicated to warrant a trial; or the trellis construction was too expensive; or the labor in pruning,

stripping and tying was too great; or cultural operations were hampered. The following methods were tried in the experiment:

Single-stem, four-cane Kniffin
Two-stem Kniffin
Umbrella Kniffin
High Renewal
Horizontal Arm
Chautauqua
Munson

These methods, in the order named, are now to be described.

METHODS OF TRAINING GRAPES IN THE CHAUTAUQUA EXPERIMENT.

The seven methods tried out in this experiment fall into a simple classification which makes conspicuous their chief characters. They may be grouped under two main heads: (1) The disposition of shoots; (2) The disposition of canes.

The Disposition of Shoots. The shoots which carry the grapes are disposed of in three ways in the various systems of pruning; shoots upright, shoots drooping and shoots horizontal. In this experiment we are concerned with but the first two. The terms are self-explanatory, but perhaps a few words of amplification are needed.

In the drooping method, the growing shoots are not tied, but droop down thruout the growing season. Usually, one less wire can be used in the drooping method than in the upright one; since the shoots need not be tied, some labor is saved. Our experiments have shown that vines with drooping shoots can be tilled more easily and with less injury. The drooping shoots, no doubt, also protect the grapes somewhat from sunscald.

In the upright method, two or more canes or arms are laid off to right and left, and carried along a horizontal wire, or are carried obliquely across two or more such wires. As the shoots grow upward, they are tied to wires above. The upright methods fall into two classes, characterized by the terms " cane renewal " and " spur renewal," according to the manner in which fruiting shoots are obtained from the vine. The cane renewal is further divided into two methods designated by the names "high renewal" and "arm renewal."

The Disposition of Canes. The canes may be disposed of in many ways in training the grape. They need not be discussed in this paragraph, since the full description of methods that follows is largely concerned with the disposition of canes. We come now to a discussion of the several methods which may be classified as follows, according as the shoots are upright or drooping:

I. Shoots Drooping:
   1. Single-stem Kniffin.
   2. Two-stem Kniffin.
   3. Umbrella Kniffin.
   4. Munson.

II. Shoots Upright or Horizontal:
   1. High renewal.
   2. Horizontal arm.
   3. Chautauqua.

Single-stem Kniffin.

The Single-stem Kniffin is the best representative of the methods in which the shoots droop. In New York it is used more than any of the other drooping methods, and seems to be gaining in popularity. It is necessary, first of all, to describe the trellis used in this method.

The posts used in the Single-stem Kniffin method should be eight or eight and one-half feet in length. These stand twenty-four feet apart in the row. Two wires are necessary, the lower being from three to three and one-half feet above the ground, while the upper one is from two to three feet above the lower wire. The wire may be annealed or galvanized. Some prefer No. 9 wire and others No. 10.

The young vines are trained as follows: The plants when set, as in all other methods, are cut back completely to a spur of two or three buds. Since young plants usually have two or more canes at setting time, a selection of the cane that is to be spurred from which may come the future trunk must be made carefully. The choice should be the cane that is most centrally located on the head and the one which grows most upright. The vines should be set so that the lower bud of this spur is just above the ground level. The first season at least one shoot springs from each bud, while others may start from below the ground. At the close of the first growing season

it is usually best to cut back again the best cane to a single spur of two or three buds. At the close of the second season the vine will have developed two or more strong canes. The strongest and best of these is now selected to form the trunk. This cane is pruned just long enough to reach the lower wire of the trellis which must be established at this time for the various Kniffin methods. In case no cane is of sufficient length to reach the lower wire, the pruning will be that of the previous year, or else, a small willow or strong twine may be used to bridge the gap.

The cane selected to make the trunk is now tied to the lower wire, the best tying material being a small willow. The willow holds the cane firmly to the wire and reduces the danger of girdling to a minimum. It is important that the trunk thus formed be straight and upright since vineyard management is carried on much better where there are straight, upright trunks. One node should project beyond the point of tying. When the shoot from the primary bud has reached the length of a few inches, it is broken off, permitting the shoot from the secondary bud to grow in order that the sap flow may be continued thruout the growing season to the top of the cane. By thus keeping the top of the cane alive, there is less danger of the young trunk breaking at the point of tying.

From this cane, during the season, shoots will put out from each bud, those nearer the top of the cane being the stronger. This is well for it is the cane arising just below the lower wire that is to form that part of the trunk between the two wires. The shoots lower down, within a foot or so of the ground, should be broken off shortly after they start. The remaining shoots may be allowed to bear some fruit.

The pruning at the close of the third season consists in the selection of a cane arising just below the lower wire for a continuation of the trunk. This is pruned long enough to reach somewhat above the upper wire. Two canes, arising somewhat below the lower wire, are pruned back to six or eight buds, and tied to the right and left along the lower wire. The bases of these canes become the more or less permanent arms from which originate the fruiting canes of the future.

The vine, at the beginning of the fourth year, consists of the completed, permanent trunk reaching the upper wire, to which it is tied, and the two canes tied to right and left along the lower wire. The

trunk is also loosely tied to the lower wire. All shoots below the lower wire should be removed early in the season, all but three or four of those between the two wires are removed. These are allowed to bear fruit.

The pruning at the close of the fourth year consists in cutting back on each cane at the lower wire one of the canes of the current season to a spur of two or three buds, and one cane of from six to eight buds. The cane that has grown nearest the trunk on the canes of the past season is usually spurred, while the cane just outside is pruned long for tying to the wire, leaving on the lower wire two short arms each carrying a spur and a cane. The pruning at the top wire consists in cutting away all growth except two canes arising just below the wire and two or three canes to spurs of two or three buds, one on the left and the other on the right. These insure fruiting canes for the following year. The canes usually consist of from eight to ten buds each and are tied down to right and left on the top wire. They are twisted around the wire a few times before tying to increase the stability of the cane.

The vine now goes into the fifth growing season completely established as to the method of training. Subsequent pruning consists in cutting away all but four canes, two at the lower wire and two at the upper wire, with sufficient spurs at each level to supply fruiting canes for the year following. If the old arms lengthen too much, new arms may be obtained by making use of the canes that spring directly from the trunk at the desired position.

By pruning the four canes long or short, the amount of fruiting wood may be regulated to the capabilities of the plant. To equalize the sap flow in the two wire levels the upper canes should be pruned longer than the lower. Another method of accomplishing this end is to establish a secondary trunk a foot or more below the lower wire from which the fruiting canes are taken. When this division of the trunk is near the ground, the method is called the Y-stem Kniffin.

As the vineyard attains age, it is often found that a new trunk must be developed on certain vines. The new trunk may be developed from a sucker which is tied to the old trunk for a support. While this renewal is being made, the vine must be pruned closely. In most cases there need be no total loss of crop while the new trunk is being developed.

The Two-stem Kniffin.

The Two-stem Kniffin differs from the Single-stem Kniffin in having two trunks instead of one. The trellis is the same. One stem in the two-trunk method reaches to the lower wire on which two canes are laid while the second stem reaches the top wire on which two more canes are laid off. Spurs to furnish the fruiting canes for the following season are left at the head of each of the two trunks. The pruning for the two first years in the two-stem method is identical with that of the one-stem method. For the third year, however, two canes instead of one are carried to the lower wire level but no shoots are allowed to grow from them except in the region near the wire level or at least not over a foot below. In the pruning for the fourth season one stem is continued to the top wire, and becomes the permanent trunk. The other stem, which reaches near to the lower wire is pruned back to two canes and two or more spurs. The canes are tied to the wire as in the one-stem method. By the fifth year the vine has been brought to its permanent shape when two canes are retained at, or near, the upper wire on the longer trunk. The pruning for the years to follow is identical with that of the Single-stem Kniffin.

The Umbrella Kniffin.

The Umbrella Kniffin has its framework formed in a somewhat different manner from the single-stem and the two-stem methods. In the Umbrella the mature vine consists of a trunk carried to the upper wire from the head of which two canes are carried diagonally down to the lower wire and tied. The pruning for the first two years is identical with that of the other Kniffin methods. In the third year the pruning usually varies a little though it may follow closely that of the Single-stem Kniffin up to the fifth year.

In the pruning of the fifth year the arms and canes at the lower wire are entirely cut away while the canes at the upper wire are pruned long enough to be bent down to the lower wire at an angle of 45 degrees from the vertical stem. In addition, spurs for the fruiting wood of the year following must be maintained. The formation of a Y terminal of the trunk is very desirable with this method, as the canes are more readily bent without breakage. With a Y at the extremity of the trunk which does not quite reach the upper wire it is well to carry the canes up and over the wire and then down to the

lower wire. The head of the vine can be held firmly in place at the upper wire either by the use of willows or soft twine. The trunk is tied loosely to the lower wire. A third wire is desirable with this method. It is placed between the upper and lower wire but a little nearer the lower wire. It serves to brace the cane, and greatly reduces the risk of breakage of the cane when it is heavy with fruit. It is also desirable in years when the canes make so short a growth that they will not reach the lower wire.

The Munson

The Munson or Modified Wakeman is not well adapted to commercial vineyards in the North, but may often be used to advantage by the amateur grower. Some varieties of grapes grown by this method give much better fruit, and the grapes are protected from the sun and the pestiferous robins, which all but ruin the grape crop in some parts of New York. Maturity of fruit is, however, delayed thru excessive shading. This method, too, is, possibly, the most decorative of the several methods.

In making the trellis for the Munson method, posts are set as for the Kniffin methods, after which a quarter-inch hole is bored through each post at a height of four feet from the ground and in the direction in which the wire is to run. A No. 9 or No. 10 wire is then put through the holes from one end of the row to the other, and fastened securely. Two pieces of 2x4s, each two feet long, are now slotted on the two inch face midway between the ends to a depth of an inch. The slot needs to be only wide enough to allow the wire to pass through it. On the opposite face, two slots are sawed in each 2x4, two inches from the ends. These need be only wide enough to carry the wire. The pieces are now ready to be placed on the end posts as arms for supporting two of the three wires that the trellis carries. The middle slot is placed over the wire that runs through the posts, so that the wire serves as a support for the arm which is securely nailed to the post. In addition, the arms are braced by running a piece of wire around each end and then down and around the post. Staples will hold this wire brace securely to the post. Similar arms are made for the intermediate posts, but instead of the 2x4 stock, 1x4 is heavy enough if the rows are not of too great length. These arms are fastened to the posts as are the end arms. Two more wires must be added to complete the trellis. These are placed on the two sides

of the middle wire and into the slots sawed on the upper faces of the arms. They are held in place by winding the brace wires that steady the arms once or twice around them. The trellis, when complete, consists of a center wire through the posts with two others a little above and on either side of it. The center wire serves as a partial support of the cross arms, which, in turn, support the lateral wires. The three wires require tightening each spring.

If the vigor of the vine permits, a cane is brought to the middle wire in the third season and firmly tied. All but a few of the shoots arising near the top of the canes are broken off early in the season. The following year, all but two canes and two spurs are pruned away. The canes are chosen near the head of the vine, and are so placed that one can be tied in either direction along the middle wire. These vary in length from eight to fifteen buds each, depending upon the vigor of the vine. When the vine has become well established, two more canes may be taken off near the head, and tied diagonally out to the lateral wires. The main purpose of the lateral wires, however, is to afford attachment for the tendrils as the shoots grow to them and consequently afford a support for the loaded canes later in the season. The canes are twisted around the middle wire, and tied with soft twine, no other tying being necessary. The trunk may be renewed much as in the Kniffin methods.

The High Renewal.

In the High Renewal, all wood older than two years is cut back at each pruning, altho some grape growers maintain two short arms from the trunk. The trellis is much the same as in the drooping methods except that, in the high renewal, three wires are always required. The lower wire is placed from eighteen to twenty-four inches above the ground; the second twenty inches above the lower; and the third twenty inches above the second.

In this method the vines are cut back to two buds at each pruning for the first two years. Under average conditions the trunk is formed by the end of the third year. All that has been said relative to the selection of the cane for the trunk in the other methods applies here. The cane selected is carried to the lower wire and there firmly tied. It is often more important with this method than with the Kniffin methods to select a straight trunk. All shoots from below or near the ground should be removed early in the season. Those that

arise near the lower wire should be loosely tied to the wire with raffia, rye straw or soft twine. Thus, the liability to injury from cultural practices is eliminated. By the fourth year, the head of the vine is formed. This should be established a few inches below the lower wire. Two canes that have grown from the stem near this position are now selected, and laid to the right and left of the head.

In the Keuka Lake region where this method is the common one, the canes are tied to the wire with willows. In addition, at least two spurs of two or three buds length are retained to furnish fruiting canes for the following year. With the Concord the canes may carry ten buds each for the first two or three years and a larger number later, but the Catawba is not able to carry over five or six buds to each cane unless very vigorous. With this method of training the shoots, of necessity, arise at the lower wire, and, if not supported, would droop into the row. Hence, as soon as the growth reaches the middle wire, it is tied loosely with rye straw, raffia or twine. Again when the growth is sufficient, the shoots are tied to the upper wire. Summer tying of the shoots is almost continuous during the heavy growth of the vine pruned under this method.

The following year, all the wood is again cut away except two or three canes that have developed from the basal buds of the fruit canes of the previous year or those that have put out from the spurs. If the third cane is retained, it is tied to the middle wire. Spurs are again provided close to the head for future renewals. If the same spurs are maintained for a number of years, they become so lengthened that the canes arising from them are above the wire, and cannot be tied as well as those which come from below the wire. In order to make it easy to lay down the canes along the wire for tying, many growers form a Y just below the lower wire, one branch of which carries the cane and spur for the right side, the other for the left side of the trunk. It is possible to renew annually the entire aerial part of the vine with exception of the trunk and its two divisions. By bringing a shoot from the ground up the trunk may be renewed without the loss of even a single crop.

The Horizontal Arm.

The trellis for this method is the same as for the High Renewal. The vine in its first years is handled just as with the High Renewal. A trunk to the lower wire with two permanent arms running to the

right and left constitute the framework from which the fruiting wood is maintained. This stage is reached in the third year. The canes which later become the permanent arms of the horizontal arm spur method should have a greater number of buds than those annually utilized in the High Renewal, the aim being to establish arms of sufficient length that the allotted trellis space may be fully utilized. If the number of buds carried by this greater length of cane be greater than the root system is capable of supporting, a number of the newly developing shoots may be broken out, or the fruit clusters removed, and the shoots allowed to grow. The remaining shoots are tied first to the middle wire and, as they elongate, to the upper, rye straw, raffia or twine being suitable tie material. At this time, the vine trained to this method is much as if pruned to the High Renewal, except that the arms are longer, and there are no renewal spurs on the head of the vine.

The pruning for the fourth season consists in cutting away close to the arm several of the canes of the past years' growth, and cutting back the remainder to spurs of two or three buds. The number of spurs retained is dependent on the vigor of the vine. This can be gauged by using bud counts as a basis. If one of two Concord vines, pruned differently, but growing under similar soil conditions and of equal vigor be pruned to 30 fruit buds, it is a fair assumption that a like number of buds will be supported in the other. This number of buds would be carried by not over 15 spurs. The spurs may be distributed at intervals of from five to ten inches along the arms. The distance apart of the spurs is dependent upon distance apart of the vines in the rows which in turn influences the length of the arms. In addition, variation in the vigor of varieties affects the length of arm, and may make it necessary to shorten the spacing of the spurs.

Two or more shoots will put out from each spur, and these are tied to the middle and third wires as fast as their development permits. The vine trained to this method presents the same general appearance in the fourth growing season as it did in the third, with the exception of the two-year-old spurs.

In the pruning for the fifth season, the canes that have grown from the upper bud of each spur are cut away, providing the basal bud of each has thrown a well matured cane, along with the upper part of the spur, and at the same time the cane that has grown from the basal bud of each spur is cut back to two buds. Thus the vine

presents the same general appearance as in the fourth season, namely, a trunk with two arms leading from it along the lower wire, and upon which are several spurs of one year wood. The spurs will in turn throw shoots that bear the fruit of the current year. There is however, a slight difference in the appearance of the spurs, the lower part of each being the growth of two years, while the spur proper is one year old.

The pruning of subsequent years follows that of the previous, but there is a tendency in the spurs to lengthen from year to year; as they lengthen they become more and more crooked, hence it is well to cut them away entirely every few years and grow others from shoots that arise directly from the arms. If buds do not arise from the old arms, adventitious buds may be forced by reducing the number of fruiting spurs in any year. It is also possible to renew the arms from time to time, but this should be anticipated a year or more in advance so that renewal spurs may be retained at or near the head of the trunk. When desirable, renewal canes may be brought out and may be tied down to the lower wire as when the foundation was laid for the vine framework. When this stage is reached, the old arms are cut away.

The Chautauqua.

This method is similar, in one respect, to the High Renewal in that it belongs to the Cane Renewal rather than the Spur Renewal group. It is similar to the Horizontal Arm Spur method in that more or less permanent arms are maintained. The framework of the vine is identical in both. In one the canes are tied to the upper wire, while in the other the canes are cut back to spurs, and the resultant shoots are tied up. The trellis for this method is very similar to that of the Arm Spur and High Renewal, except that two wires are more commonly employed than the three of the other two named. Three wires in this method are desirable and often essential, especially in wind-swept locations, and also in seasons following scant wood growth. The third wire eliminates the "stringing up" of the canes, that is, the use of twine in bridging the space between a cane, that is too short to reach the upper wire, and the wire.

In most vineyards the lower wire in this method is 18 to 20 inches from the ground, and, if but two wires are used, the second is about 34 inches above the lower. If three are employed, the upper wires

are about 20 inches apart. The treatment for the first two years is much the same as the other methods except that, in the case of quite vigorous vines, a cane (the future trunk) may be carried to the lower wire the second year. If this be done at that time, all but two or four shoots near the head should be broken off early in the season. The remaining shoots should be tied loosely to the lower wire. If willows are not available for tying the trunk to the lower wire, twine should be used. If girdling results it is of little consequence, as long as it is above the point from which the shoots have started. The head of the vine should be below the lower wire, rather than at or above it. With vines of average vigor, the trunk is formed in the third year.

Assuming that the trunk is formed the third season, the pruning for the fourth year consists in cutting away all but two or three canes and two or three spurs situated on or near the head. The canes, one to the right of the head and the other to the left, are twisted once loosely around the lower wire, and then carried obliquely to the middle or upper wire as the vigor of the vine permits. Some vineyardists who make use of but two wires space the upper wire at a less distance than 34 inches during the first few years, and then later raise it to the required height. The canes, whether carried to the middle or upper wire, are tied tightly with small wire, twine or willows on the windward side of the wires. If three wires are in use, and the canes are carried to the upper wire, they are loosely tied to the middle wire. If vigor permits, a third cane is led from the head obliquely to the middle or upper wire. If the canes that are wrapped around the wire arise a few inches below the lower wire, the head of the vine is advantageously fixed for future years.

At the close of the fourth season, numerous canes will have grown from the nodes of the two canes that were tied up in the spring of that year. Three or four of these are to carry the fruit crop of the fifth season. A part of each cane that produced these, that is, those that were twisted around the lower wire is to serve for some years as a permanent arm. The pruning at this time consists in cutting back each of these canes to within three feet of the head, and in cutting from them entirely all but three or four canes that have grown the past season. At intervals along the arms, two- or three-bud spurs are retained which, probably, will furnish the fruiting canes a year later. The canes retained should be of sufficient length to reach

from the arm, when tied to the lower wire, to the middle and upper wires. One or more spurs should be left on the head for emergencies. All growth that has developed between the ground level and the head should be cut away.

Before growth starts, the vine is tied to the trellis as follows: the shortened arms, now of two seasons' growth, are twisted loosely once or twice around the lower wire, one to the right, the other to the left. Assuming that the arms have been pruned so that each carries two canes, these are led to the middle or upper wire obliquely, and there tied with fine wire, twine or willows. A vine at this stage consists of a trunk reaching to a few inches below the lower wire, two arms extending from it, along the lower wire, from each of which two canes arise, one about 12 inches to the right or left of the trunk, and the other 18 inches further out on the arm. These four canes are to bear the fruit of the year.

Shoots will develop from the buds of the nodes of the canes and spurs, so that the vineyardist has considerable leeway in his selection for the future seasons, altho those from the basal buds of the canes of the past season are, as a rule, short and poorly matured while those from the apical buds are well grown and ripened. If the buds on the spurs have produced desirable canes, the pruning is much simplified, for a cane can be chosen from three or four of the spurs that are properly spaced on the arm and tied up, while at the same time canes that have grown from adventitious buds on the arms may be spurred for the following year. Often the canes of the past season, located nearest the head, will have grown well matured canes of sufficient length that the old arms may be entirely cut away, and the cane of two years previous laid down to the wire as an arm, with the past season's growth furnishing the fruiting canes. Some growers thus renew the arms each year.

For trunk renewal, a well-grown shoot coming from the ground is selected, and in the first two seasons may be tied loosely to the old trunk. In the third season, the head is formed as has been described for the initial establishment of this method of training. While the new vine is being formed, the old aerial portion should be pruned more severely, that the best growth may be made by the renewal. As soon as the framework has been attained, the old trunk, with its radiating arms, is entirely cut away. In this renewal there need be little loss of crop.

The Hudson River Umbrella.

This method is somewhat used in the Hudson River Valley, and, while it bears the name Umbrella, it is not a true Umbrella. The trellis is similar to that for the Kniffin methods, with the exception that the wires are closer together. The upper wire is placed at the height of the Kniffin methods, five to six feet above the ground, but the lower wire is from 20 to 24 inches below. The trunk is carried to the upper wire in a manner similar to the Kniffin, but from this point it assumes a form similar to a reversal of the Chautauqua Arm method. To illustrate, from the trunk reaching to just below the upper wire, two canes lie along it, one to the right and the other to the left. The shoots droop and attach themselves to the lower wire. At the pruning for the following season, all but from four to six of the canes are cut away, while several are pruned to spurs. The canes are pruned to sufficient length to reach to the lower wire when carried down somewhat obliquely to it, where they are firmly tied. The vine at this stage presents exactly the reverse appearance of the vine in the Chautauqua method. Subsequent pruning follows closely that of the Chautauqua method, a combination of cane and spur renewal being practiced.

RESULTS FROM THE SEVERAL PLATS.

FRUIT YIELDS.

Table I gives the fruit yields on the acre basis of 680 vines for each plat for the years 1911 to 1918 inclusive and also the eight-year average. It must be kept in mind that other results than yield are of prime importance. Thus, one method by reason of its cane disposal, might bring forth as much fruit but of better quality; another

TABLE I.— FRUIT WEIGHTS IN PRUNING AND TRAINING EXPERIMENT.

Mode	1911	1912	1913	1914	1915	1916	1917	1918	8-yr. Av.
	Tons	*Tons*	*Tons*	*Tons*	*Tons*	*Tons*	*Tons*	*Tons*	*Tons*
Munson (Modified Wakeman)	3.50	4.00	2.20	4.40	5.40	2.99	4.50	1.59	3.57
Single-stem Kniffin	5.44	5.20	2.21	5.16	4.62	3.23	3.57	1.89	3.91
Chautauqua	5.20	5.13	1.88	5.06	4.52	2.39	3.62	1.75	3.69
Umbrella Kniffin	4.38	4.52	2.22	4.35	4.42	2.60	3.91	1.76	3.52
High Renewal	3.80	2.65	2.01	4.65	2.97	1.55	2.88	1.42	2.68
Two-stem Kniffin	4.59	4.59	1.46	4.99	3.77	2.55	2.80	2.02	3.34
Horizontal Arm Spur	3.11	2.94	....	....	....	....	....	....	3.02
changed to Hudson River Umbrella	....	....	....	3.94	5.37	2.96	3.74	2.75	3.75

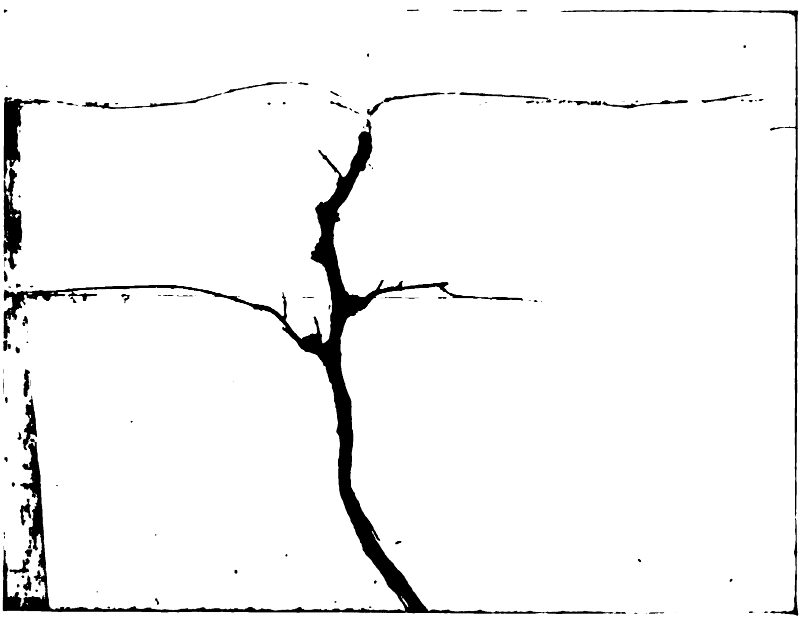

Plate XXXV.— The Single-stem Kniffin Method of Training.
Upper, before pruning. *Lower*, pruned and tied.

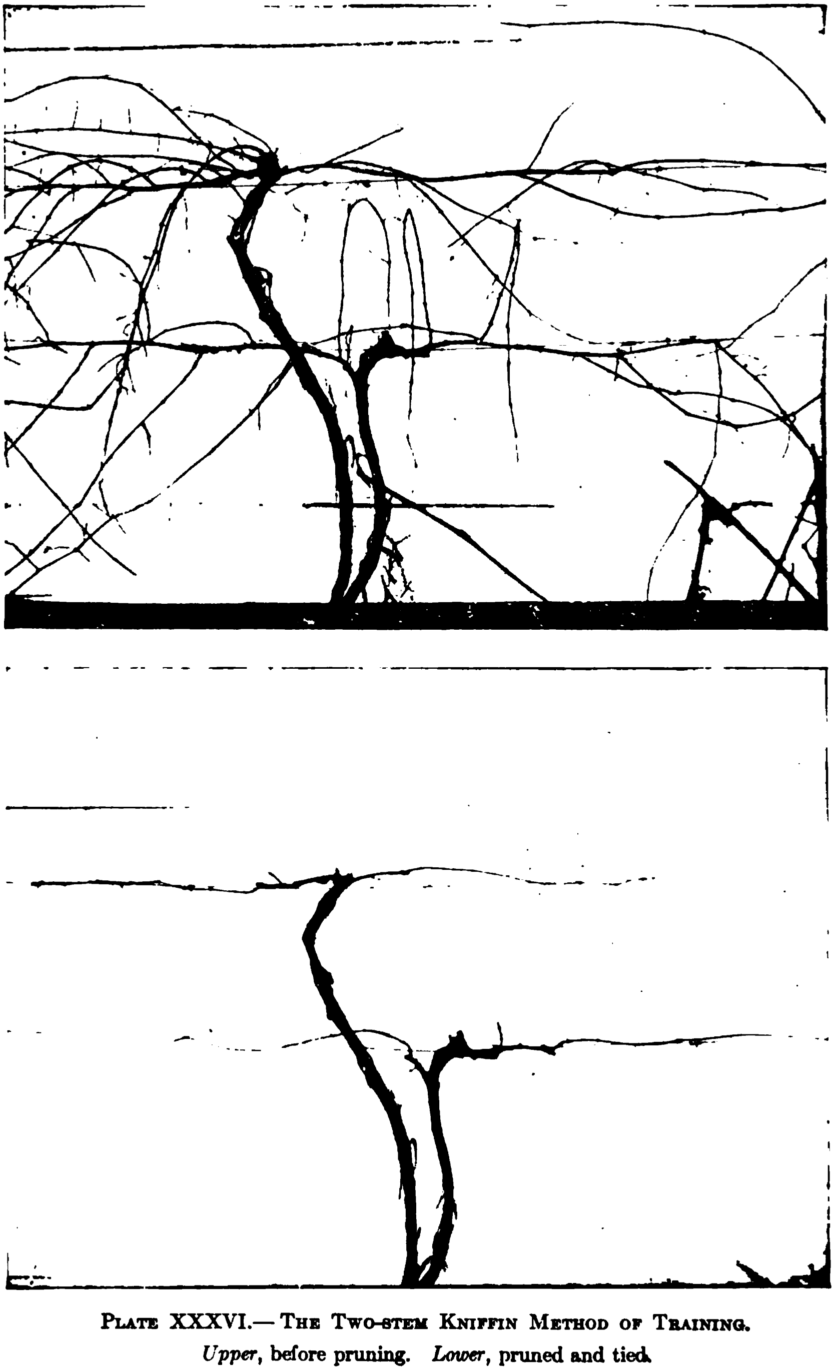

PLATE XXXVI.— THE TWO-STEM KNIFFIN METHOD OF TRAINING.
Upper, before pruning. *Lower*, pruned and tied.

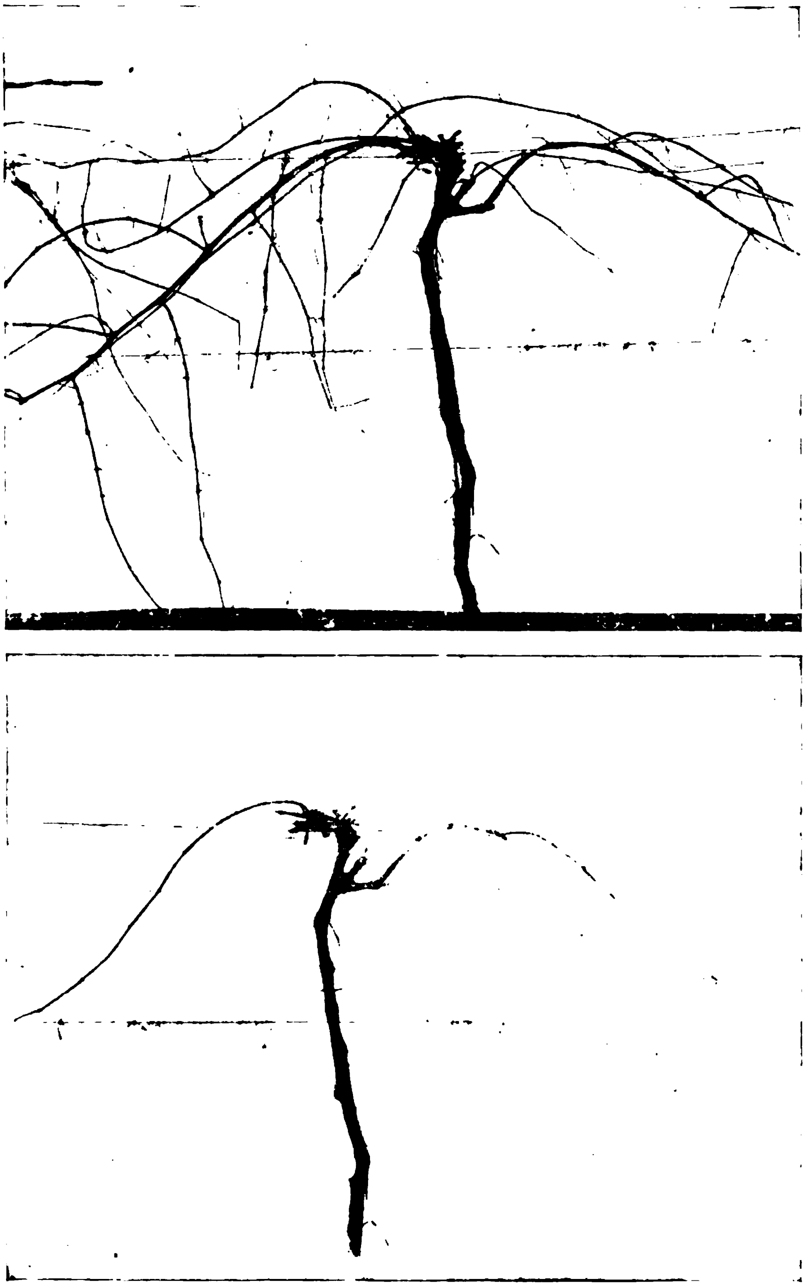

PLATE XXXVII.— THE UMBRELLA KNIFFIN METHOD OF TRAINING.
Upper, before pruning. *Lower*, pruned and tied.

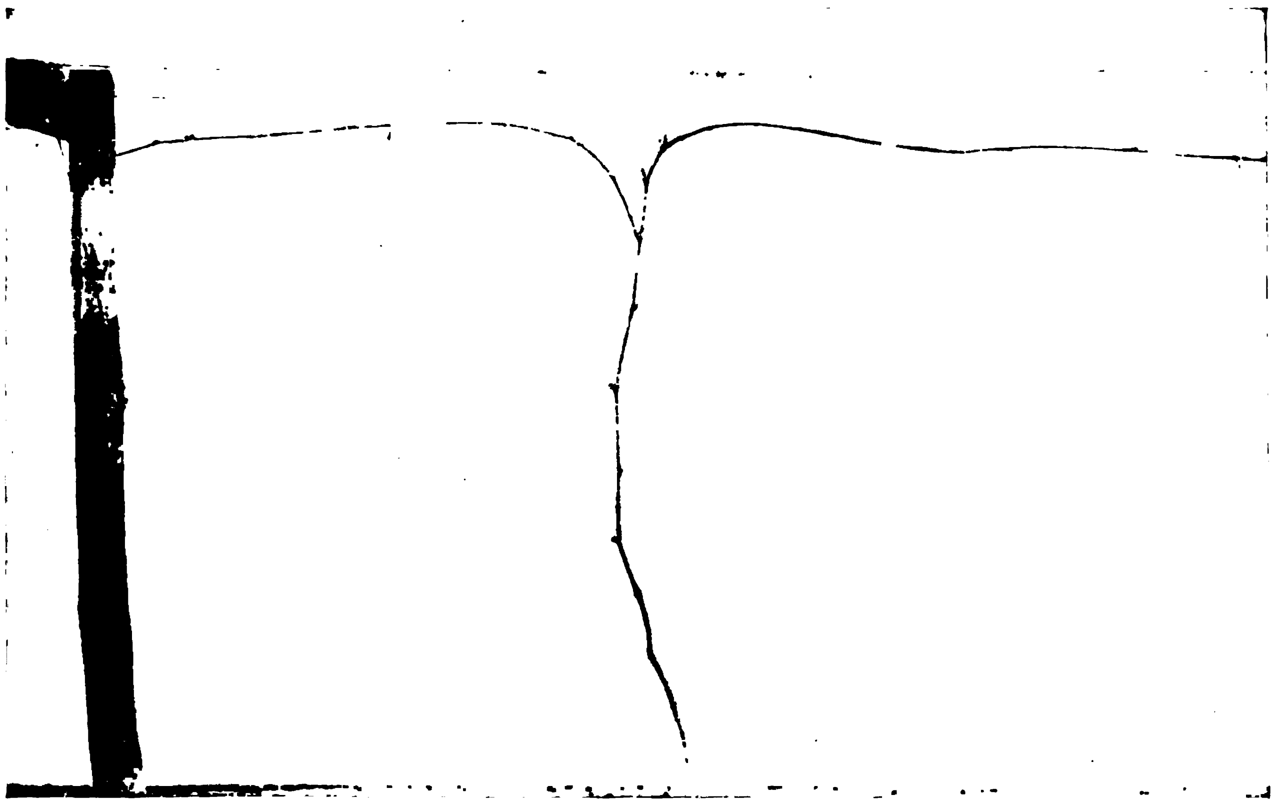

Plate XXXVIII.—The Munson Method of Grape Training.
Pruned and tied.

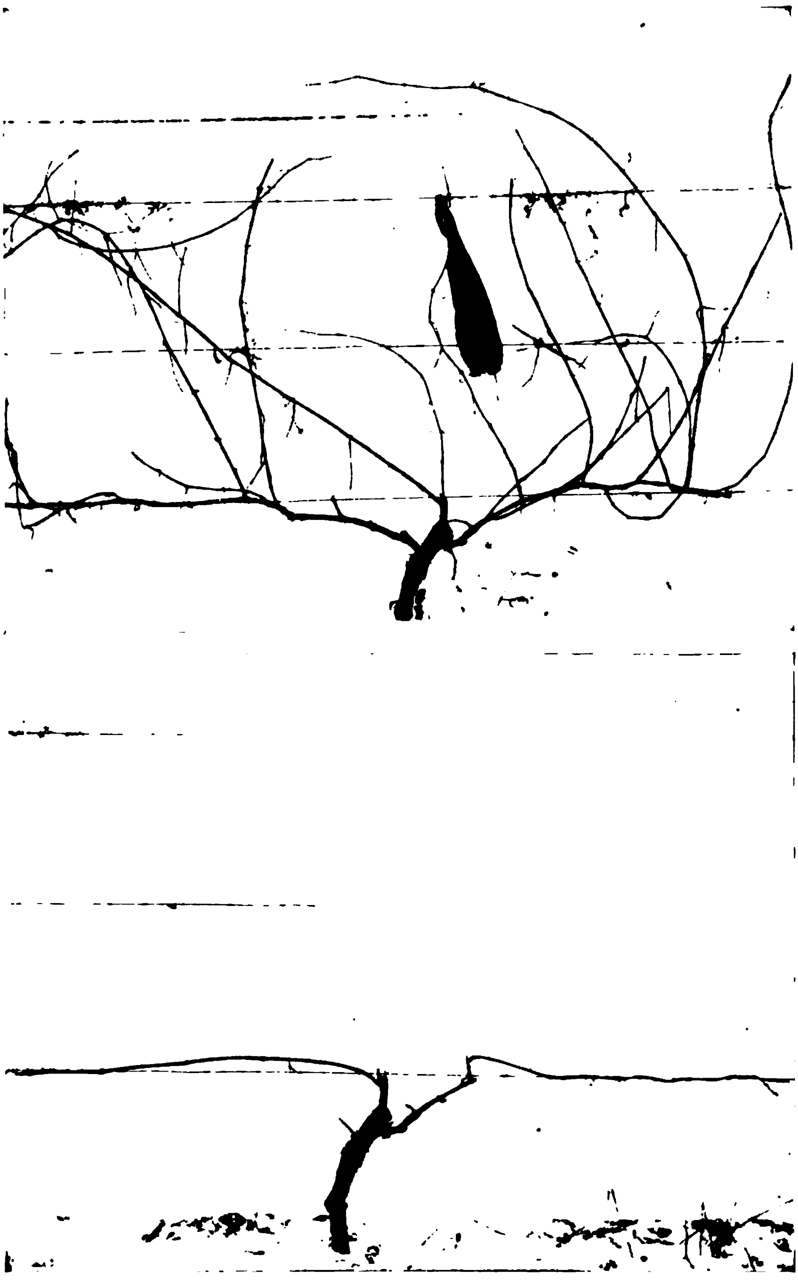

PLATE XXXIX.— THE HIGH RENEWAL METHOD OF TRAINING.
Upper, before pruning. *Lower*, pruned and tied.

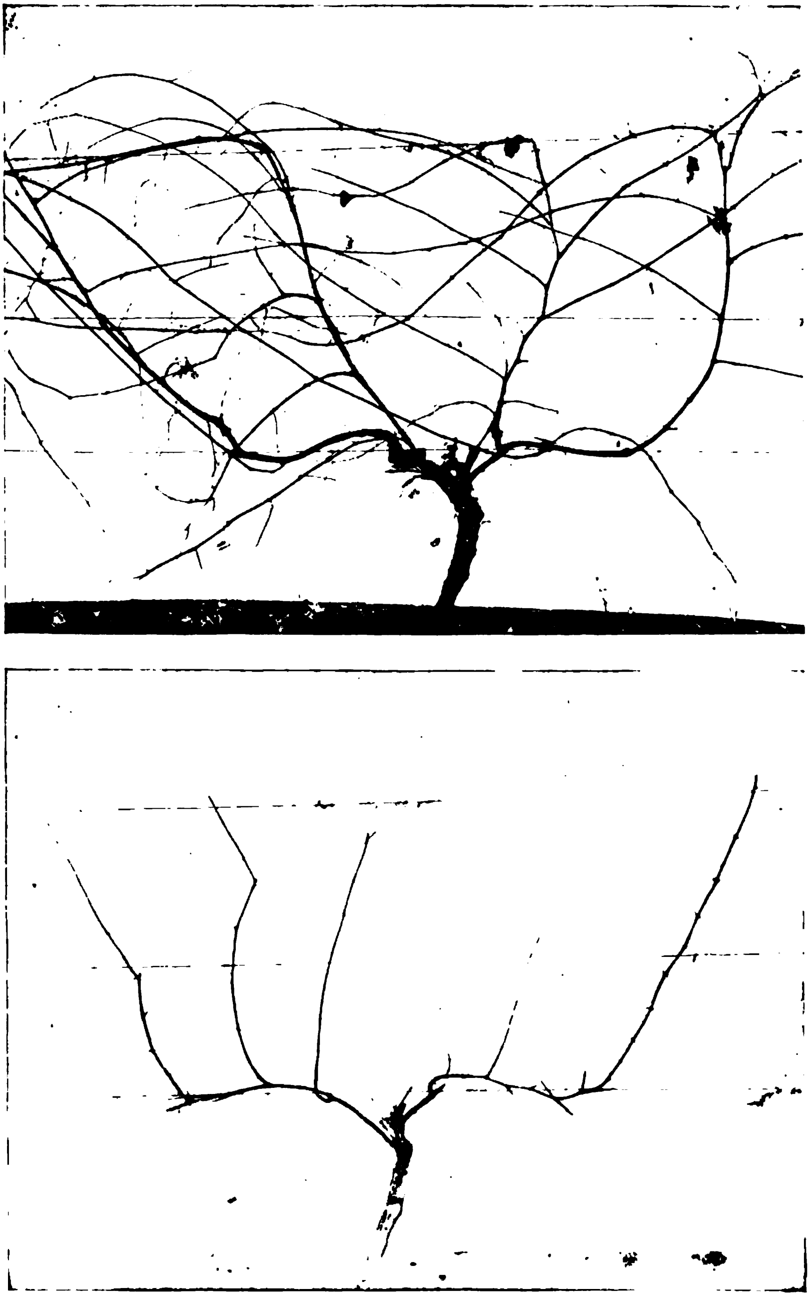

PLATE XI.— THE CHAUTAUQUA METHOD OF GRAPE TRAINING.

Upper, before pruning. *Lower*, pruned and tied.

Plate XLI.— The Hudson River Umbrella Method of Training.
Upper, before pruning. *Lower*, pruned and tied

PLATE XLII

Upper, The Chautauqua or Arm Method at the beginning of growth in the Spring. Illustrating the tendency to develop the best canes at the upper wire.

Lower, The Two-stem Kniffin photographed at the same time and showing a more equable development of shoot, but still showing a tendency to stronger growth at the head of the vine.

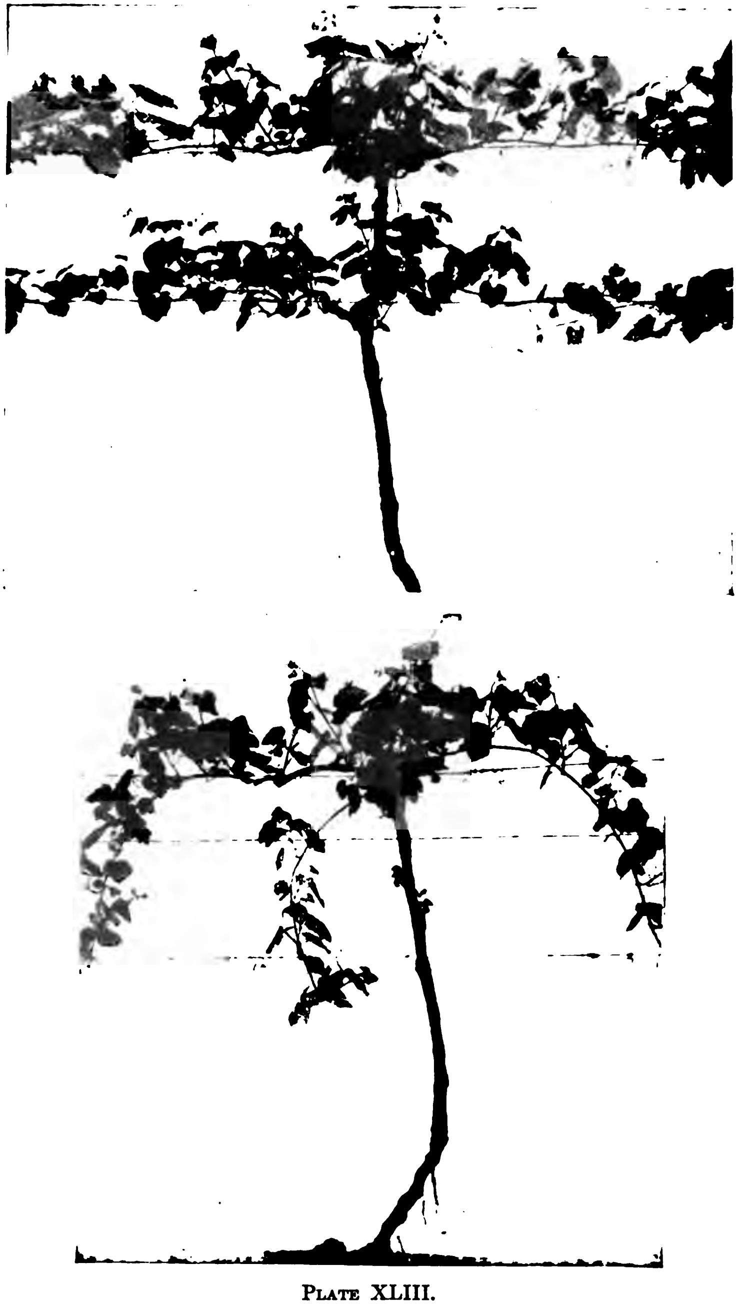

PLATE XLIII.

Upper, The Single-stem Kniffin illustrating equality of shoot growth along the entire length of the canes.

Lower, The Umbrella Kniffin illustrating again the better growth of shoot at or near the head of the vine as well as in the region of the bends of the canes,

Plate XLIV.

Upper, The High Renewal Method after the starting of growth in the Spring. Shoots not yet of sufficient length to be tied to wires.

Lower, The Hudson River Umbrella at the beginning of growth. The shoots that are now growing upright will shortly assume a pendant position. No further tying is required as the distance from the ground is so great that the new growth is kept well away.

method might possess characters that would facilitate the various vineyard operations. Some of the methods, by reason of the restriction upon their bearing wood, necessarily bear less fruit. Others have borne an excessive number of under-ripe clusters. Table I shows that the plat trained to the Single-stem Kniffin has returned the highest average for the eight years; that for the same period the Chautauqua method has been second; while, of the other methods, the High Renewal has been the lowest producing plat.

After the first two years the Horizontal Spur method was discarded, for the reason that the quality of fruit was inferior. This plat was then changed over to the Hudson River Umbrella, for which the records covering five years are given. The averages indicate that, so far as quantity of fruit is concerned, there is not much choice between the Munson, Single-stem Kniffin, Chautauqua, Umbrella Kniffin, Two-stem Kniffin, and Hudson River Umbrella. If the number of fruiting canes that are usually alloted to the Munson had been adhered to, the quantity of fruit from this method would have fallen short of the others. The construction of the trellis makes it possible to put up more than the allotted two, however. This leeway is lacking with the Arm Spur method, and, as these vines were more vigorous than those in ordinary vineyards, the tendency to rampant wood growth could not be checked thru putting up more buds.

The High Renewal, in a lesser degree, presented the same difficulty, but this was partially overcome by putting up additional canes to the middle wire. After the first two or three years it was seen that the Umbrella Kniffin would readily support an additional cane in average seasons, and thru this the yield was made to approximate that of the higher yielding plats. Thus it is fair to assume that six of the eight methods under test are of approximately equal value, so far as the quantity of fruit yielded is concerned. This is a consideration of first importance to the commercial grower, for quantity production, when the fruit is used for wine or unfermented juice, takes equal place with quality.

The low yields over all the plats in 1913-1916 and 1918 are attributable to winter injury of the buds during the preceding winter. Many buds were killed outright, while others were so injured that their clusters failed to develop normally. There is some indication that the vines trained to the drooping methods fared better than those trained to the upright methods.

Weight of Wood from Various Plats.

Table II records the weight of wood pruned each year with the six-year average, from the different methods of training. A consideration of these data shows that the Munson and High Renewal have had the greatest amount of wood pruned away over the six-year period, while the Hudson River Umbrella has had the least. The Single-stem Kniffin has had slightly less wood pruned away over the six years than the Chautauqua, which may in part account for the somewhat increased yield over the Chautauqua. However, the Chautauqua favors the development of more cane growth through the location of the canes in a more or less vertical position. A comparison of Table I with Table II indicates that all of the methods, with the possible exception of the Two-stem Kniffin, and the High Renewal, are maintaining a fair balance between the amount of fruit produced and cane growth, as indicated by the amount pruned away. It is also evident that the Two-stem Kniffin should be shorter pruned, while the High Renewal indicates that these vines should be longer pruned. The amount of wood pruned away with the Hudson River Umbrella would suggest that the vines trained to this method are just about at their limit of fruit production.

TABLE II.— WEIGHT OF PRUNED WOOD IN POUNDS PER ACRE FROM PRUNING AND TRAINING EXPERIMENT.

Mode	1912	1913	1914	1915	1916	1917	6-Yr. Av.
	Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
Munson (Wakeman)	1455	1605	1448	1748	1550	1394	1533
Single-stem Kniffin	1214	1125	1169	1426	1429	1598	1327
Chautauqua	1420	1448	1462	1557	1557	1537	1497
Umbrella Kniffin	1220	1292	1482	1435	1302	1265	1333
High Renewal	1305	1625	1554	1744	1438	1510	1529
Two-stem Kniffin	1108	1020	1425	1306	1187	1196	1207
Hudson River Umbrella	.....	.....	.....	1295	1091	1129	1172

With the pruning to the Chautauqua method, quite frequently, or at intervals of two or three years, the old arms are cut away, hence something more than the annual cane growth is represented in the wood weights.

The data from Tables I and II tend to prove that, with the exception of the vines trained to the High Renewal and Horizontal Arm Spur methods, the methods under test were about equally valuable so far as the yield of fruit and the production of wood is concerned,

with the suggestion that the Two-stem Kniffin ranks as the poorest in the latter group. It is also evident that the two first named methods are not adapted for Concord growing under conditions that favor vigorous growth.

Influence of Pruning on Fruit Characters.

One of the most important considerations in a test of pruning is a study of the influence of method on fruit characters. It is certain that the effect of method upon maturity, size and compactness of cluster, size of berry and its sugar content are as important as production.

Comparisons during the past eight years show that the fruit from vines trained to the Umbrella Kniffin have been the most fully matured; fruit from the Chautauqua, Single-stem Kniffin and the Munson are nearly as good, with not much choice between the three. In the third group falls the fruit from the High Renewal and Horizontal Arm, while fourth, and by far the least matured, has been the fruit from the Two-stem Kniffin. The better maturity of the fruit from the Umbrella Kniffin may, in large part, be due to the lessened production, but even in years of equal productivity the fruit has matured better. It can be stated as a fact, therefore, that this method does favor maturity more than the other methods tested.

For compactness and size of cluster, the Umbrella Kniffin ranks a little ahead of the Chautauqua and the Single-stem Kniffin, but the total weight of fruit from the Umbrella has been less by reason of the fewer number of clusters. There is no appreciable difference in these characters between the Chautauqua and the Single-stem Kniffin. The clusters from the Munson rank below the methods just named, but are much superior to those from the High Renewal, Horizontal Arm and the Two-stem Kniffin. The fruit from the last named method was the poorest in all respects, and that from the other two methods but slightly better. The Hudson River Umbrella yields fruit comparable to that from the Chautauqua method. The fruit from the vines trained to the Umbrella, the Chautauqua, the Single-stem Kniffin, the Munson and the Hudson River Umbrella methods, has been such that most of it would go into fancy packages, while the vines trained to the Two-stem Kniffin, the Horizontal Arm and the High Renewal, gave much that could be classed only as seconds, or even waste in some seasons.

The data as to the sugar content of the fruit grown by the various methods is too meager to warrant any definite statements. There seems to exist, however, a correlation between the height from the ground that the fruit is borne and its sugar and acid content. This is especially noticeable when the vines are trained to the Chautauqua method. Fruit from the higher half of the vine contained thirteen per cent more sugar than that from the lower half, while, at the same time, the acid content was lower. Theoretically, those methods that bring the fruiting shoots to a considerable height from the ground should favor the development of fruit of higher sugar content as well as of reduced acidity. These methods are the Single-stem, the Two-stem and the Umbrella Kniffin, the Hudson River Umbrella and the Munson. It has been noted that the fruit from the Two-stem Kniffin matured least well, therefore the influence of favorable elevation of the fruiting wood for sugar content may be nullified by other conditions such as underpruning.

Cost of the Several Methods.

We may dismiss the Munson method as a commercial possibility for the trellis construction is too complicated and expensive, and the upkeep is somewhat greater than with other forms of trellis. Time in the annual tightening of the wires also results in an increased charge. On the country estate, for arbors, and in the home garden the Munson method lends itself admirably for the training of the vigorous varieties of grapes. The position of the fruit and leaf permits good air drainage, while, at the same time, they are kept away from tools used in cultural practices. The fruit, being borne high from the ground, is kept free from disfigurement by the soil splashing upon it. Pruning is no more complicated with this method than with others of the same type, but tying and harvesting are much more difficult. The spraying of vines trained to this method must be done with a trailing hose and with more care than in any of the other methods tested. The pendant clusters, as viewed from beneath, appeal to the grape fancier, as all of the fruit is plainly to be seen.

The Single-stem, the Umbrella and the Two-stem Kniffin methods may be discussed collectively. All three possess the good points of the Munson, with some additional ones. The trellis and maintenance cost is but slightly greater than that employed for the Chautauqua, and considerably less than that for the Munson. The cost

for the Umbrella is a little greater than that of the other two, as a third wire is desirable. If three wires be utilized with the Chautauqua method, then trellis cost for the Single and Two-stem Kniffins is no greater than that for the Chautauqua. Practically the only difference in cost between the Kniffins and the Chautauqua trellis of two wires is in the additional length of post required for the former, altho the longer post is now proving a better investment even with the Chautauqua method of training.

One of the great advantages of the Kniffin methods, and this applies with equal force to the Munson, is the permanency of the canes when once tied in the spring. With nearly all of the other methods the canes are very frequently blown down during the summer, especially as the fruit nears full size and weight. Not only must much time be employed to re-tie, but the fruit from contact with the wet soil becomes discolored and bruised. In addition, the entire cane may be broken close to the arm or trunk, and thus the crop of the next season is lessened and the form of the vine changed.

Tying in the Different Methods.

Pruning to the Single-stem Kniffin is the simplest of all the methods, that of the Umbrella and Two-stem Kniffin being but slightly more complex, however. Tying requires no more skill, and, when the tying for all methods is done with twine, the time required for tying the Umbrella has proved less than for any other method. Tying in the Single-stem Kniffin has been accomplished in much less time than that of the Chautauqua or the Hudson River Umbrella, the latter two requiring about the same expenditure of time. The alternatives offered in the pruning of the Single-stem and the Two-stem Kniffin, the Munson and the Hudson River Umbrella are so varied that, from this viewpoint alone, they are of decided advantage over the Chautauqua, the Umbrella Kniffin and the High Renewal. The objectionable practice of stringing up canes, common to the Chautauqua, is eliminated in the practice of these other methods.

Vines trained to the Single-stem Kniffin, the Umbrella, the High Renewal, the Two-stem Kniffin and the Hudson River Umbrella may be more thoroly sprayed when fixed nozzles are used than in the Munson or Chautauqua. The High Renewal, of all methods, is most thoroly sprayed.

Harvesting fruit from the vines trained to the Single-stem, the Umbrella and the Two-stem Kniffin is accomplished just as easily as with the Chautauqua. Harvesting from vines trained to the Hudson River Umbrella requires somewhat more time. While the time required in harvesting is not an objectionable feature with the High Renewal vines, yet the fact that the fruit is borne in close proximity to the lower wire makes the task a back-breaking one. Of all the methods, pickers consider the Munson the most objectionable for harvesting.

The grape grower in the Chautauqua Belt is sorely tried thru the canes being blown down by frequent high winds. As previously noted, the cost of labor in re-tying is but a small part of the loss. The fruit, discolored and bruised through contact with the soil, is practically worthless, while the injury to the woody parts of the vine is a serious one. The vineyard must often be gone over five to eight times during the summer season for the purpose of re-tying. The handling of the canes blown down, as the period of maturity approaches, further tends to injure the fruit. In 1918 careful record was made of the number of canes that were re-tied with the Single-stem Kniffin, the Umbrella Kniffin and the Chautauqua methods. Table III shows the re-tying necessary for the three methods, which includes 144 vines trained to each.

TABLE III. TYING FOR THE THREE METHODS.

Method	Canes Retied June 17, 1918	Canes Retied June 24, 1918	Canes Retied July 6, 1918
Single Stem Kniffin	1	3	0
Chautauqua	2	50	43
Umbrella Kniffin	0	13	0

From the table it is seen that the Single-stem Kniffin, with but four canes blown down during the season, is least troublesome and the Chautauqua, with 104 down, is most so. This season was not exceptional for its high winds. The Two-stem Kniffin, Hudson River Umbrella and the Munson may be classed with the Single-stem Kniffin as to the permanency of the tying. The High Renewal is not at all subject to this trouble, as the canes are carried low on the

trellis. Occasionally, however, the shoots which are tied to the second and third wires break off, but this does not allow the fruit to come in contact with the soil, even if the tendrils have not fastened to the wires or to other shoots.

The options offered in pruning thru the employment of the Munson, Single-stem Kniffin, Two-stem Kniffin and the Hudson River Umbrella make any of these methods more desirable than the Chautauqua or the Umbrella Kniffin. In years of average growth, it is possible to get canes from vines trained to the Chautauqua or the Umbrella Kniffin that will reach to the upper wire with the first named, and to the lower with the last. But, as growth has been below the average during the past eight years, it has been difficult to get well matured canes of sufficient length, and, as a consequence, "stringing up" has been practised. The canes of the vines trained to the Chautauqua method, thru their nearly vertical position, develop the strongest at their extremities. These are valueless for the succeeding year, being too far removed from the head of the trunk, consequently inferior ones must be selected, while those that will not reach are "strung up." A third wire relieves the situation somewhat, but the canes, carried no higher than the middle wire, allow the growing shoots to reach the ground, and, as a consequence, they interfere with cultural operations. This is the chief defect of the Chautauqua method. With the Single-stem Kniffin, Two-stem and the Hudson River Umbrella, while canes of proper length are preferred, nevertheless the length of cane does not interfere to any appreciable extent with the pruning and later tying.

The principal defect of the High Renewal method of grape training, aside from the tendency to excess wood production with vigorous varieties, is the summer tying required. This is almost continuous from the time the shoots are long enough to reach the middle wire until near the harvest season. The number of times the vineyard must be gone over is, of course, dependent upon the rapidity of summer growth. With slow or weak growing varieties, or on thin soils, this method has proved its practicability.

Time to Prune.

For the past seven years the Station has been testing the merits of early winter compared with late winter pruning upon the time of foliation, fruit yields and wood growth. The vines in the test are

the same as those in the test of pruning methods. One row was pruned in early winter, while the other, trained in like manner, was pruned ten days to two weeks previous to leafing. The exact dates necessarily vary from season to season. The early winter pruning has been done after temperatures of eighteen degrees or lower have been reached.

Table IV shows the fruit yields during the past seven years, as well as the seven-year average for each method of training and for each of the pruning periods. Considering the data for 1912, it is seen that there are no consistent differences that favor either period,

TABLE IV.— FRUIT WEIGHTS IN TONS PER ACRE IN A TEST AS TO THE MERITS OF EARLY WINTER AND SPRING PRUNING.

Mode	1912	1913	1914	1915	1916	1917	1918	7-Yr. Av.	
	Tons	*Tons*	*Tons*	*Tons*	*Tons*	*Tons*	*Tons*	*Tons*	
Single Stem Kniffin	5.23	1.86	4.69	4.65	2.60	3.60	1.82	3.49	Winter Pruned
	5.23	2.50	5.57	4.59	3.74	3.57	1.90	3.87	Spring Pruned
Chautauqua.......	5.14	2.06	4.82	4.99	2.10	3.43	1.66	3.45	Winter Pruned
	5.06	1.70	5.27	4.40	2.65	3.77	1.80	3.52	Spring Pruned
Umbrella Kniffin..	4.60	2.12	4.25	4.01	2.24	3.57	1.70	3.21	Winter Pruned
	4.40	2.33	4.45	4.87	2.92	4.21	1.80	3.57	Spring Pruned
High Renewal.....	2.00	2.49	4.42	3.02	.08	2.82	1.42	2.45	Winter Pruned
	3.20	1.52	4.93	2.89	2.10	2.92	1.30	2.70	Spring Pruned
Two-Stem Kniffin.	4.70	1.68	5.00	3.47	2.58	3.00	1.90	3.20	Winter Pruned
	4.40	1.23	5.47	4.04	2.51	2.58	2.14	3.19	Spring Pruned
Hudson River Umbrella.	....	....	3.70	5.03	2.60	3.40	2.61	3.47	Winter Pruned
	....	....	4.15	5.47	3.20	4.04	2.87	3.96	Spring Pruned

while the same tonnage for each, with the vines trained to the Single-stem Kniffin, indicates that the fluctuations with the other methods for the two periods are due to other causes than time of pruning. In 1913 the data are equally inconclusive, certain rows yielding higher for the early pruning, while others did not yield so well. For the season of 1914, the spring-pruned rows showed an increase ranging from one-fifth to nine-tenths of a ton. Again in 1916 the yields are, with one exception, higher from the spring pruned rows. The increases range from fifty-five to one and fourteen hundredths tons, while one row of the spring pruned fell below the early winter pruned by seven-hundredths of a ton. The data for 1917 and 1918 show no consistent differences that can be attributed to time of pruning. A consideration of the seven-year average for the rows pruned for com-

parison at different periods, shows, with one exception, a slight gain in the fruit harvested from the spring-pruned vines. The gains are probably due to the ability of the pruner to select the best matured canes in the spring after they have been subjected to the low temperatures of winter, while at the earlier pruning this immaturity was not so evident.

In table V are given the weights of the wood pruned from each set of rows. A study of the figures indicates that the period of pruning has had little or no effect upon the amount of wood growth. In four instances the early winter-pruned and the spring-pruned have had the same amounts of wood cut away, while the six-year average is very inconclusive, as one set seems to favor the spring-pruned, and another the winter-pruned.

TABLE V.— WEIGHT OF PRUNED WOOD FROM THE PRUNING AND TRAINING EXPERIMENT VINEYARD, GIVING THE COMPARISON BETWEEN THE ROWS PRUNED IN EARLY WINTER AND SPRING.

Mode	1912	1913	1914	1915	1916	1917	6-Yr. Av.	
	Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	
Single-stem Kniffin	1319	979	1210	1390	1258	1455	1235	Winter Pruned
	1102	1271	1128	1462	1605	1741	1385	Spring Pruned
Chautauqua	1564	1578	1462	1557	1510	1510	1530	Winter Pruned
	1276	1319	1462	1557	1605	1564	1465	Spring Pruned
Umbrella Kniffin	1428	1360	1502	1408	1258	1306	1377	Winter Pruned
	1013	1224	1462	1462	1367	1224	1292	Spring Pruned
High Renewal	1550	1836	1557	1788	1367	1510	1601	Winter Pruned
	1061	1414	1550	1700	1510	1510	1462	Spring Pruned
Two-stem Kniffin	1414	1102	1339	1435	1197	1197	1280	Winter Pruned
	802	938	1510	1176	1176	1197	1133	Spring Pruned
Hudson River Umbrella....	....	....	1166	1176	959	986	1072	Winter Pruned
	....	....	....	1414	1224	1272	1303	Spring Pruned

Considering the data, it is evident that pruning of the Concord in this locality may be done with the same results, any time after leaf fall, preferably, however, after several days of low temperatures, and extending till the beginning of active sap flow. No ill results have been noted with vines pruned after the buds had begun to unfold.

In view of the fact that the Concord in the Chautauqua Belt has been severely winter killed in bud at least three seasons during the past ten years, it would seem advisable to delay pruning until after the low temperatures of winter have passed, and this especially

following a season that has been unfavorable for maturing bud and wood. With large acreages, this is an impossibility, unless sufficient men are available for pruning. Spring pruning allows of better judgment in pruning to canes that are in best condition, those having the least number of injured or dead buds.

Late spring pruning suggests itself as being most desirable for those varieties that require a long growing and maturing season, and hence are tender in bud. It also commends itself for those varieties that possess the inherent character of tenderness in bud, aside from length of season required for maturity.

REPORT

ON

INSPECTION WORK.

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L. L. VAN SLYKE, *Chemist*.

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REPORT ON INSPECTION WORK.

REPORT OF ANALYSES OF SAMPLES OF COMMERCIAL FERTILIZERS COLLECTED BY THE COMMISSIONER OF AGRICULTURE DURING 1919.

[Text of this Bulletin, No. 467, December, 1919, is omitted, since the data cease to have value before the Annual Report can be distributed.— W. H. JORDAN, *Director*.]

INSPECTION OF FEEDING STUFFS, 1919.

[Text of this Bulletin, No. 469, December, 1919, is omitted, as the data cease to have value before the Annual Report can be distributed.— W. H. JORDAN, *Director*.

SEED TESTS MADE AT THE STATION DURING 1918.

[Text of this Bulletin, No. 462, June, 1919, is omitted, as the data cease to have value before the Annual Report can be distributed.— W. H. JORDAN, *Director*.]

APPENDIX.

I. Popular Editions of Station Bulletins.

II. Periodicals Received by the Station.

III. Meteorological Records.

POPULAR BULLETIN REPRINTS.

MISSING HILLS IN POTATO FIELDS: THEIR EFFECT UPON THE YIELD.*

L. G. COLLISON.

Loss in yield.

Potato growers, generally, appear to take it for granted that the effect of missing hills or "skips" in potato fields is to lower the yield. Accordingly, efforts are made to obtain as nearly a full stand of plants as may be possible. Concerning the amount of the loss, opinions differ widely. Some assume that a missing hill is a total loss. Others hold that a large part of the loss is made up by the increased yield of the adjacent plants. Probably, the amount of the loss varies considerably with the variety, the distance between hills, and the cultural, soil and weather conditions; but there seem to be few experimental data upon which to base an opinion.

An experiment.

During the past season, therefore, an experiment was conducted for the purpose of throwing light on these questions. An experimental plat was planted to potatoes of the variety Sir Walter Raleigh, and the arrangement of the hills was as indicated in the scheme below. The missing hill, or skip, is indicated by the capital O between the hill groups.

$$\begin{array}{cccccccccccccccccccc} X & X & X & X & O & X & X & X & X & O & X & X & X & X & O & X & X & X & X \\ a & a' & b' & b & & a & a' & b' & b & & a & a' & b' & b & & a & a' & b' & b \end{array}$$

FIG. 1.—SHOWING METHOD OF PLANTING IN ROWS.

In each group a and a' were halves of the same tuber; likewise, b' and b were from the same tuber. Under parallel conditions a and a' should give the same yield; also, the yield of b' should equal that of b. But if missing hills affect the yield of adjoining plants, a should outyield a' and b outyield b'.

* Reprint of Popular Bulletin No. 459, May, 1919. The complete bulletin is reprinted on p. 189.

A check plat. also, was planted after the same scheme, except that the skips were eliminated.

Results. After equalizing conditions as far as possible, a careful calculation of results was made. It was found that in weight of total yield the exterior (or *a* and *b*) plants outyielded the interior (or *a'* and *b'*) ones by 23.2 per ct. on the average. Accordingly, the answer given by the experiment is that, under conditions such as obtained in this experiment, the loss from missing hills is offset to a considerable extent by the increased yield of adjoining plants. In the case of a skip containing a single missing hill the two adjoining plants (one on either side) together make up 46.4 per ct. of the loss in total yield and a little more in yield of marketable tubers. Skips of more than one hill are, probably, a total loss except for 46.4 per ct. of the yield of one plant. Furthermore, the number of tubers per plant was 20.7 per ct. greater for the exterior plants than for the interior ones, and the tubers were of larger average size.

APPLICATION OF RESULTS.

The statement made above, that the loss from missing hills probably varies considerably with the variety, the distance between hills, the character and fertility of the soil and the cultural and weather conditions, should be repeated and emphasized. The fact must not be overlooked that the results obtained in this experiment apply only to a single set of conditions, namely, to the variety Sir Walter Raleigh, planted 15 × 36 inches, on heavy clay loam soil of medium fertility, under weather conditions such as prevailed at Geneva in 1918. They are not of general application. However, let us consider how they may be used when the conditions are such as to make them applicable.

Since the effect of a missing hill is to increase the yield of adjoining plants, it follows that a partial stand gives a larger yield than is indicated by the stand. In other words, a stand, of let us say 80 per ct., will give more than 80 per ct. of the yield of a full stand. If this larger percentage be designated " stand value " we may say that a stand of 80 per ct. has a stand value of more than 80 per ct.

Stand value and corrected yield. In plat experiments with potatoes the yields of plats which differ much in stand cannot be properly compared until the yields have been corrected for full stand. In such cases it is highly important to know how to correct yield for full stand. The formula frequently used, namely, corrected yield $= \frac{\text{actual yield}}{\text{stand}}$, is clearly incorrect, because it is based on the assumption that missing hills are a total loss, which

is not true. The formula which should be used is, corrected yield $(CY) = \frac{\text{actual yield}}{\text{stand value}}$. For the calculation of stand value we may use the formula, $SV = 1 - \frac{m - 0.464s}{n}$ in which m is the number of missing hills per acre, s the number of skips* per acre and n the number of hills per acre in a full stand.

Let us see how these two formulas may be applied in the solution of the following problem: On a tenth-acre plat of Sir Walter Raleigh potatoes planted 15 × 36 inches, there were 100 skips with a total of 232 missing hills. The actual yield was 20 bushels. What was the stand value and what was the yield per acre corrected for full stand?

In the above problem $m = 232 \times 10 = 2320$; $s = 100 \times 10 = 1000$; and $n = 11600$. Substituting these values in the formula for stand value, we have $SV = 1 - \frac{2320 - 464}{11600} = 1 - \frac{1856}{11600} = 0.84$ or 84 per ct. Hence, $CY = \frac{200}{0.84} = 238.1$ and we have the answer: the stand value was 84 per ct., and the yield corrected to full stand was at the rate of 238 bushels per acre.

From the above discussion it will be seen that it is impossible to correct the yield for full stand when only the number of missing hills is known. It is necessary to know both the number of missing hills and the number of skips.

It is to be hoped that other experiments of a similar kind may be made for the purpose of deriving stand-value formulas for other sets of conditions. While it is probably impossible to derive a formula having general application, it seems to the writer quite possible to obtain formulas which will give results approximately correct when applied to particular sets or combinations of conditions.

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* A skip is a series of consecutive missing hills.. It is assumed that, when potatoes are planted 15 × 36 inches, skips of more than a single hill are a total loss except for 46.4 per ct. of the yield of one hill; that is to say, the adjoining plants on either side make no more use of a large skip than of a small one, and plants in adjoining rows are not influenced. This may not be strictly true

NOTES ON NEW YORK PLANT DISEASES, II.*

F. C. STEWART.

Notes made on many diseases

For the most part, the Station Botanist is occupied with the investigation of a few important problems —chiefly plant disease problems. Nevertheless, he has opportunity for making observations and brief studies on numerous other diseases of plants. Whenever there comes to his attention anything in this line which is new or interesting, notes are made upon it. Such notes are classified according to host plants upon which the diseases occur, and filed for future reference. In the course of time there is a considerable accumulation of notes. They are then sorted, and such of them as appear worthy of permanent record are prepared for publication in bulletin form. The complete bulletin, of which this is a review, contains notes on about forty plant diseases occurring in New York. A considerable number of these are of interest, chiefly to plant pathologists and so need not be mentioned here. Others, of more or less economic importance, will be treated briefly.

Apple fruit-pit

Fruit-pit of apples is a disease the symptoms of which are circular dead brown spots on the surface of the fruit and small pockets of dead brown tissue underneath the skin. On October 25, 1913, a quantity of each of 120 varieties of apples grown on the Station farm was picked and put into storage. Only perfect specimens were selected because it was intended to use these apples in the fruit exhibits to be made by the Station during the following winter. During the first three weeks of storage the temperature of the storage room was kept below 50 F. by the use of ice. After November 15 no artificial methods of heating or cooling were employed.

A careful examination of each variety was made on December 23. It was then discovered that about 50 percent of the apples of one variety, Lehigh Greening, were severely affected with fruit-pit, while none of the other 119 varieties grown and stored under parallel

* Reprint of Popular Bulletin No. 463, December, 1919. The complete bulletin is reprinted on p. 214.

conditions were affected. This indicates that Lehigh Greening is very susceptible to fruit-pit; also, that fruit-pit may develop in storage.

Petiole rot of spotted arum

The spotted arum is subject to a destructive disease in which the leaf-stalks become rotten. The disease first attacks the leaf-stalk at or below the surface of the soil. The affected tissue may be either moist and water-soaked in appearance or dry and brown. In either case the discoloration extends rapidly from the base of the leaf-stalk to its summit. In plants attacked during the early part of the season the discolored tissue is very moist, soft and foul smelling and the epidermis may be easily removed by rubbing with the fingers. In plants attacked after the middle of July the diseased tissue is usually brown and quite dry. Whether the rot assumes the wet or the dry form appears to depend more upon the age of the leaf than upon the state of the weather.

Almost from the first appearance of the disease the leaf-blade begins to change color, and, by the time the rot has reached the summit of the leaf-stalk, the blade is yellow. The whole leaf now falls to the ground and decays. A prominent character of the disease is its tendency to run up one side of the leaf-stalk while the opposite side remains sound. The disease is remarkably rapid in its action.

Inoculation experiments with pure cultures have shown the cause of the disease to be the bacterium *Bacillus carotovorus* which also causes a soft rot of carrots, cabbage and other vegetables.

Defoliation of butternut trees.

Butternut trees often cast their leaves prematurely in consequence of the attacks of a fungus thought to be the same as that causing a spotting and dropping of black walnut leaves. Affected butternut leaves show irregular brown spots of all sizes from a mere speck up to about three-eights of an inch across. On the tips and margins of the leaves the spots frequently coalesce and form large dead, brown areas. Fruit bodies of the causal fungus occur on both surfaces of the leaves. They may be detected with a hand lens. Under a microscope they are found to contain numerous colorless, two-celled, sickle-shaped spores. Usually, September is the season at which the effects of the fungus are most conspicuous; but almost complete defoliation of some trees has been noted as early as the latter part of July.

Cabbage blackleg

Apparently, this troublesome fungus disease of cabbage is becoming quite prevalent in some parts of New York, particularly in Ontario county where it has been reported repeatedly as causing serious damage during the past three or four years. The variety most frequently affected is Allhead Early. In some cases the diseased plants are scattered irregularly thru the field, but it often

happens that many plants in one row are dead or dying with the disease while plants in the adjoining row are all healthy. This leads to the belief that the disease is frequently contracted in the seedbed and disseminated with the seedlings. Other investigators have proven that the disease may originate in infected seed. Also, it appears probable that a diseased seedling may infect others with which it comes in contact in the process of transplanting. The common practice of bunching the seedlings and placing the roots in water preparatory to transplanting is highly favorable to such infection. This may account for the marked tendency of the disease to appear in certain rows and not in others.

The danger arising from the use of infected seed may be largely avoided by soaking the seed for fifteen minutes in a 1-1000 solution of corrosive sublimate, or immersing it for twenty to twenty-five minutes in a 1-200 solution of 40 per ct. formaldehyde, followed by washing in clear water.

Black leaf-speck of cabbage

From widely separated places in the State the Station has received complaints of a cabbage disease having the following symptoms: The white leaves all thru the head (sometimes, also, the outer leaves) are plentifully sprinkled with minute black specks varying in size from a mere point to the size of a small pinhead. Many of them resemble fly specks. Most of the spots are broadly elliptical, their major axes lying parallel with the larger veins of the leaf. In location, the spots bear no definite relation to the veins. Some are seated on the veins, others on the tissue between the veins. Some are visible from both sides of the leaf, others from one side only. Sometimes the spots occur singly, sometimes in irregular groups. With the aid of a hand lens it may be seen that the surface of the spots is slightly sunken (cover illustration).

This disease occurs both on cabbage in the field and in storage. Fortunately, it is not common. The cause is unknown. It may, perhaps, be of bacterial origin, but it is not caused by any fungus.

Storage rot of carrots

Where carrots are stored during winter in large quantities, either in cellar storage or cold storage, one of the regular troubles is a form of rot in which areas of softened tissue are covered with a copious growth of white mold. Several times during the past twenty years carrot growers on eastern Long Island have complained to the Station of heavy losses due to this rot. Cold storage houses in Rochester, also, have occasionally reported loss from the same cause. The white mold is the fungus *Sclerotinia libertiana* a well known plant parasite. It may be identified by the large, black, somewhat irregular bodies (sclerotia) which are produced in considerable numbers on the decaying carrots. Our studies have convinced us that this fungus, by itself, may cause extensive decay of stored carrots; but, usually, there is associated with it an active

soft rot bacillus, probably *Bacillus carotovorus*. Two or three other species of fungi, also, are frequently associated with the decay.

Catalpa leaf spot and twig blight

From time to time the Station has been called upon to explain the nature of a twig blight and leaf spot of young catalpa trees, the symptoms of which strongly suggest fungi or bacteria as the cause. We have often been puzzled by these troubles, but have finally reached the conclusion that the twig blight and a part, at least, of the leaf spot are caused by an insect, the catalpa midge.

In August and September short shoots of the current season's growth are found to be dead, blackened and shriveled. This is the twig blight. The leaf spot takes the form of circular, dead brown spots one-eighth to one-fourth of an inch in diameter.

Cherry leaf spot

Leaf spot is a common fungus disease of cherries in which the leaves first show numerous small brown spots, then turn yellow and fall prematurely. In wet seasons leaf spot is very prevalent and destructive in New York. In many orchards of sour cherries the trees are almost completely defoliated before the fruit is fully ripe. Many trees are so much weakened that they afterward die of winter injury.

For the control of cherry leaf spot the Wisconsin Station recommends plowing under the fallen leaves before the blossoms open, and spraying two or three times either with bordeaux mixture, 3-3-50, or lime-sulfur, 1-40. The first spraying should be made soon after the petals fall, and a second one two weeks later. If a third spraying seems necessary it should be made as soon as the fruit is harvested. In each application add arsenate of lead to the fungicide at the rate of three-fourths to one pound of the powder, or one and a half to two pounds of the paste, to each fifty gallons.

Clover anthracnose

In June, 1914, the Station Botanist was asked to investigate the unhealthy condition of some red clover fields in the vicinity of Seneca Castle. In one field of several acres the crop of hay was nearly ruined. The clover stems were still alive, but most of the leaves were dead and black, and many had fallen. Two or three other fields on the same farm were injured in a similar manner, but less severely. Severe attacks of the same disease occurred also at Geneva, Newark and South Lima. The trouble was due to an anthracnose caused by the fungus *Gloeosporium caulivorum*.

Further study of the disease was made at Geneva in June, 1917. In this case the disease was in an early stage. Leaves here and there were hanging limp beside their stems because their petioles were soft rotten. While some of the hanging leaves were dead and black, the majority were still green. Owing to the weather being showery, they had not shriveled as quickly as they would have

done had it been dry. Altho this field became thoroly infected early in the season, the damage done was not great. A fair crop of hay was harvested. More destructive outbreaks were reported that year from Seneca county and McConnellsville.

As usually found, the stems and leaf stalks of diseased plants show black streaks or elongated, black, sunken spots. Undoubtedly, this disease is of more frequent occurrence and greater importance in New York than is indicated by the few cases here recorded. Altho it has been reported as occurring at several points in the United States, the American literature of the disease is very meager.

Currant anthracnose

Currant anthracnose is a fungus disease in which affected leaves first show minute brown spots, then turn yellow, and fall prematurely. Leaf spot is a somewhat similar currant disease, but the spots are larger, commonly one-eighth inch in diameter. Both diseases are of common occurrence in New York. Frequently, they occur together. On the whole, anthracnose is considerably more abundant and important than leaf spot. The virulence of anthracnose varies greatly from year to year. It may be epidemic one season, and almost wholly absent the next. Apparently, the damage done by anthracnose and leaf spot is not as great as one might expect from the amount of defoliation which they cause. Altho both diseases may be controlled by spraying, either with lime-sulfur solution or with bordeaux mixture, it is doubtful if spraying is a profitable operation on the average.

It has been observed repeatedly that the variety Victoria, tho not immune, is notably resistant to anthracnose.

Fomes root rot of currant

Fomes ribis is a woody, yellowish-brown bracket fungus which grows around the crowns of currant plants. In New York it is not common, but occurs occasionally. Often the mass of fungus is a foot in diameter, and clings to the currant canes so tenaciously that a hammer and chisel are necessary to remove it. One would naturally suppose that such a fungus would be detrimental to the affected plants. However, it appears that such is not the case. Altho the fungus is clearly parasitic on its currant host, it is not seriously injurious to it. The presence of the fungus in a currant plantation need cause no alarm.

Currant fruit drop

The dropping of currant berries when partly grown is the cause of much loss. This trouble is comparable to the "June drop" in apples. It is widespread, and occurs more or less every year. Losses of one-third of the crop are not uncommon. The green berries may drop at any time before they are about three-fourths full grown. In severe attacks, the ground underneath the bushes is conspicuously littered with the fallen berries. Invariably, the dropping is from the ends of the clusters. In extreme cases nearly or

quite all of the berries of a cluster may fall; and even in mild attacks the ends of the fruit stems are bare.

It is quite generally conceded that weather conditions are, in some way, responsible for fruit drop, but there is much diversity of opinion among fruit growers as to the particular kind of weather which induces it. Probably, anything which suddenly checks the growth of the fruit soon after it is set may cause it to drop; for example, excessive heat, drought, a sudden drop to low temperature, or a long period of cold, rainy weather.

Fruit drop is not due to imperfect pollination. Currants are self-fertile. Moreover, we have known severe attacks of fruit drop to occur in seasons when the weather conditions at blooming time were ideal for cross pollination. In fact, it appears that an unusually heavy setting of fruit is likely to be followed by an unusually large amount of fruit drop.

No means of preventing the fruit drop of currants is known.

Powdery mildew on currants

The fungus of gooseberry powdery mildew, *Sphaerotheca mors-uvae*, occasionally attacks currants in nurseries. Usually, the damage done is negligible; but, occasionally, slight injury results. It attacks the fruit, leaves and young wood at the tips of the shoots, covering them with a conspicuous growth of fungus which is white when young and cinnamon brown when mature. Both red and black varieties of currants may be affected. It is unnecessary to apply any treatment for the disease.

Sunburn of currants

When a period of very hot weather follows a period of wet weather and rapid growth, the fruit and leaves of red currants are liable to become sunburned. The leaves show large, irregular, dead, brown spots. The ripe berries first become light in color with the appearance of having been scalded. Later they shrivel.

Sunburn is especially to be feared when the bushes carry a heavy load of fruit. The rapid increase in the size and weight of the berries as they ripen causes the canes to bend outward and downward. Then the tender leaves and berries on the interior of the bush become suddenly exposed to the sun. Should bright, hot weather occur while the bushes are in this condition a large percentage of the fruit may be ruined by sunburn. Such trouble may be largely avoided by picking a part of the fruit early to relieve the canes of a part of their load.

Anthracnose of elm

This disease attacks, chiefly, young trees of the English elm in nurseries and Camperdown elms of all ages and in all kinds of situations. In the nursery it may cause extensive defoliation. Affected leaves show numerous small, reddish brown spots and irregular, dead, brown areas on their upper surfaces; while beneath

they are thickly dotted with conspicuous heaps of white or cream colored spores of the causal fungus.

Yellow leaf of elm

In common with several other kinds of trees, Camperdown elms are liable to become affected with a non-parasitic yellow-leaf disease which causes them to shed a part of their leaves during June. Affected leaves first turn yellow without any spotting, then become brown and shriveled. Usually, they fall before changing color from yellow to brown. Yellow leaf is most severe when a long period of wet weather in May is followed by dry, hot weather in June. Altho the quantity of fallen leaves is often considerable, the health of the trees is not likely to be seriously impaired; but the litter which they make is objectionable on lawns.

Box tree "protector" causes injury

In the summer of 1910 the trunk of an elm tree on the Station grounds was encased in a "protector" consisting of a tight, wooden box about twenty inches square by six feet high to protect it from injury by animals. The tree was a thrifty one with an uninjured trunk ten inches in diameter.

In the spring of 1912 the tree showed pronounced symptoms of ill health. Soon after the leaves started they stopped growing, and early in the summer the tree died. Upon removing the box "protector" it was found that the bark was dead over a portion of the trunk. There were patches and strips of dead bark alternating with areas of live bark. The injury extended from the ground upward to a height of about eight feet. A part of the injury, at least, was a year old, for some of the wounds had healed. The dead bark was permeated by the conspicuous white mycelium of an unidentified fungus. Also, two species of boring insects were working in the bark; but Mr. Parrott, the Station Entomologist, expressed the opinion that they were not responsible for the trouble.

While there is much uncertainty concerning the immediate cause of the injury—whether winter injury, fungus, or borers—the indirect responsibility of the box "protector" seems to be clear. It appears that the close boxing of elm trunks is a dangerous practice. Tree protectors should be so constructed as to admit light and air freely to all sides of the trunk; otherwise they may do more harm than good.

SPRAYING LAWNS WITH IRON SULFATE TO ERADICATE DANDELIONS.*

M. T. MUNN.

SUMMARY.

Experiments made at the Station during the past eight years demonstrate that dandelions may be eradicated from lawns, at relatively slight expense and without material injury to the grass, by proper spraying with an iron sulfate solution. Ordinarily, four or five applications are required. The first spraying should be made in May just before the first blooming period. One or two others should follow at intervals of three or four weeks; and, finally, one or two more in late summer or fall. During the hot, dry weather of mid-summer spraying should be discontinued because of the danger of injury to the grass. A conspicuous blackening of the lawn which follows each application soon disappears if the grass is growing vigorously. Of the other common lawn weeds, some are killed while others are but slightly injured by the spraying. Unfortunately, white clover, also, is killed. Spraying should be supplemented by the use of fertilizers and the application of grass seed in the spring and fall of each year. With proper management, it is necessary to spray only about every third year in order to keep a lawn practically free from dandelions.

The cutting-out method of fighting dandelions is laborious and ineffective unless the greater part of the root is removed. Shallow cutting, unless done frequently, is worse than none at all, because each cut-off root promptly sends up one or more new plants.

Tests of certain after-treatment measures in the form of reseeding, liming of the soil, and fertilization with commercial fertilizers and stable manure, used in conjunction with the spraying operations, gave results which serve highly to recommend their use either singly or in combination on lawns.

A study of seed production in the common dandelion shows it to be parthenogenetic, that is, capable of producing viable seeds without fertilization of the ovules by pollen.

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* Reprint of part of Bulletin No. 466, December, 1919. The complete bulletin is reprinted on p. 246.

CONCLUSIONS AND RECOMMENDATIONS.

OUTLINE OF LAWN TREATMENT.

Eternal vigilance is the price of a good lawn free from weeds. The proper time to commence the fight against weeds is when the lawn is first made. Care should be taken to secure a thick, thrifty growth of grass at the start. Attempts to establish lawns on poor soil illy-prepared usually fail. After the lawn is established constant care is necessary to maintain it in a thrifty condition which will serve as a protection against weeds.

The measures most frequently used for the eradication of weeds from lawns are: (a) digging them out with a knife or spud; (b) heavy reseeding and fertilization to crowd them out; and (c) the use of chemical sprays to kill the foliage. The last-named method is the cheapest and as effective as any; but complete success requires the use of all three methods and some others.

The dandelion and certain other weeds may be eradicated from lawns, without injury to the grass, by proper spraying with iron sulfate solution. However, the weeds will soon return unless supplementary measures are employed. Unfortunately, there is no escape from the menace of dandelions seeding on adjacent grounds, because one has no control over the premises of his neighbors. Nevertheless, the occasional plants which survive spraying should be prevented from seeding by digging them out or by applying gasoline, kerosene, or dry iron sulfate to their crowns. The lawns should be mowed frequently, watered in dry weather, well fertilized, and the bare spots reseeded. Whenever the dandelions reappear in considerable numbers it will be necessary to again resort to spraying.

CUTTING DANDELIONS.

Cutting off dandelions below the crown with a knife or spud is not only laborious but ineffective unless practically the entire root is removed or the foliage completely removed several times during the season, so that the plant has no opportunity to store up reserve food in the root. Shallow digging, unless done frequently, is worse than no digging because the root, when cut off, sends up from one to several new plants and the final result is a more profuse growth of dandelions. Deep digging, whereby practically the entire root is removed with a spud, stiff-bladed knife, asparagus knife, chisel, or other special tool,

is recommended as a means of removing the few plants which survive spraying.

SPRAYING LAWNS.

Spraying with iron sulfate solution will usually prove effective when carefully, persistently and intelligently done.

NUMBER OF TREATMENTS AND TIME OF MAKING THEM.

Our experiments indicate that at least three (or, usually, five) thoro applications during a season are necessary to eradicate the dandelions from the average lawn in this state. To be the most effective, the spraying should be repeated as soon as the dandelion plant has regained new foliage and just before it is full grown — usually when the leaves are three or four inches long. This forces the plants to use up their reserve food stored in the roots, and eventually starves them. On the Station lawns, which were exposed to dandelion seeds from adjoining untreated lawns, it was found necessary to spray every second or third year, and to supplement the spraying with other control measures.

The time of application appears to be important. In our tests, the best results were secured when the first application was made in early spring after the central blossom buds were formed, but before blossoming. The first application should be followed by two or three later ones at intervals of three to four weeks during the spring growing season, and one or two others in late summer or fall. The last application should be made late enough in the summer or fall to prevent the plants from recovering before the close of the growing season.

A suitable day for spraying is one on which there is little wind and slight probability of rain for several hours. The sky may be either cloudy or clear. A heavy dew the following night is advantageous. Spraying should be discontinued during periods of drought in midsummer, when the grass is inactive and soil is very dry. Serious injury to the grass may result from spraying at such times. As far as possible, it should be arranged to spray two or three days after mowing and to mow two or three days after spraying.

STRENGTH AND QUANTITY OF SOLUTION, AND MANNER OF APPLICATION.

The spray solution is prepared by dissolving one and one-half or two pounds of iron sulfate (also called copperas and green vitriol)

in each gallon of water. The weaker solution appears to be entirely satisfactory, and is probably the one to be preferred. Used at this strength, the quantity of iron sulfate required for a single application is approximately 175 pounds per acre, or four pounds per thousand square feet of lawn. A gallon of the solution will cover about 375 square feet. Iron sulfate for spraying purposes is usually offered for sale in the granular or "sugar" form, which is readily soluble in water. It is comparatively inexpensive, costing, usually, from one to two dollars per bag of one hundred pounds. Since it corrodes metals, the solution should be prepared in wooden or earthenware vessels.

Experience has demonstrated rather conclusively that the effectiveness of the spray solution upon the dandelions depends, to a considerable extent, upon the manner in which it is applied. The best results are secured when the solution is applied in the form of a fine, mist-like spray well driven down among the foliage. While fairly satisfactory results may be expected when the solution is applied judiciously with a sprinkling can, it is recommended that some form of a spray pump be used. The kind of outfit selected should depend upon the size of the area to be treated. For small lawns a compressed-air sprayer, knapsack sprayer, or good bucket pump with brass cylinder, and equipped with a fine nozzle will be found satisfactory; while for large lawns a sprayer mounted on wheels is desirable. For very large areas (parks, roadsides, etc.) a power-driven field or orchard sprayer will be found most practical. A lead of hose at least 80 to 100 feet long should be used on the power outfits. In any case, the nozzle should be capable of delivering a fine mist-like spray which will drift evenly over the foliage, and the area should be sprayed evenly, avoiding the drenching of any particular part. One of the new type spray-guns attached to a power sprayer will be found to facilitate the work very materially when it is desired to cover a large area quickly and evenly.

The spray solution should be strained thru a fine strainer or two thicknesses of cheesecloth to remove any particles which would clog the nozzles.

CAUTION.

On stone, cement, metals, and cloth, iron sulfate solution produces a conspicuous yellowish-brown rusty stain which is extremely difficult

to remove. Accordingly, care should be taken to avoid getting any of the spray on one's clothing or on sidewalks, building foundations, monuments, curbstones and the like. Even the dragging of the wet hose across stone or cement sidewalks will stain them. In our experiments, when working around sidewalks and buildings, we have found it convenient to use a screen made of cloth tacked over a light wooden frame (3 by 6 feet). A helper is required to hold the screen in position and move it from place to place as needed.

After using, the sprayer should be washed out thoroly with clean water to prevent serious rusting. The working parts of the spray pump should be kept well oiled.

AFTER-TREATMENT MEASURES.

Our experiments and experience demonstrate that it is necessary to supplement the spraying operations with at least two after-treatment measures, namely, fertilization and reseeding.

FERTILIZATION.

The fertilization of lawns is essential in order to produce a thrifty growth of grass and dense turf for a protection against the encroachment of weeds. In our experiments, five methods of fertilization, in the form of surface applications, were tested in conjunction with spraying. Briefly, they are as follows:

(1) Spring and fall applications of bonemeal at the rate of 1000 pounds per acre, the fall application giving the best results.

(2) The application of slaked lime at the rate of 1000 pounds per acre. No noticeable response was secured from this treatment.

(3) The application of nitrate of soda at the rate of 100 pounds per acre in the spring after active growth had begun, and again in summer. This gave good results in the form of increased growth of the grass.

(4) The application of a complete commercial fertilizer in the fall.

(5) The use of well-rotted stable manure applied in the fall, and the coarse material raked off the following spring.

The results seem to indicate rather conclusively that the average lawn will require some form of fertilization to quicken grass growth and heal the turf after the dandelions and other weeds have been

killed out.[3] When well-rotted stable manure free from weed seeds cannot be obtained, perhaps the best course to follow would be to use a liberal quantity of complete commercial fertilizer in the fall after spraying and apply ground bone during the following two years.

SEEDING.

The renovation of lawns by heavy reseeding with grass seed or grass seed containing a little white clover, to thicken the turf and crowd out the dandelions, has been reported as having given good success in some cases.

Following the use of the spray solution in our experiments, it was found quite necessary to reseed the scars or bare spots in the turf left by the dead weeds. For this purpose a mixture of equal parts of Kentucky blue grass and redtop grass seed was used. This was sown on the sprayed lawn, and well raked into the bare spots, after which a dressing of compost was applied. The success attained by this method seems to warrant the following recommendation: Keep at hand, in a dry place, a supply of grass seed mixture containing equal parts of Kentucky blue grass and redtop grass seed known to be quite free from weed seeds. The two kinds of seed should be purchased separately and mixed at home. The prepared lawn mixtures upon the market are usually of very poor quality. They should never be used unless known by test to be composed of pure fresh seed. The home-mixed seed should be sown on the lawn in the spring (April or May) and again in September following spraying, and well raked into the bare spots left by the weeds. A satisfactory seeding requires five pounds of this mixture for a lot 50 x 100 feet, or one ounce per 100 square feet, at each application. If no spraying is to be done the following season, it is often advisable to add four ounces or more of white clover seed to each five pounds of the mixture. White clover responds quickly and aids in forming a dense growth over bare places where weed seeds may lodge and germinate.

[3] The subject of lawn fertilization is fully discussed in U. S. Dept. Agr. Farmers' Bulletin No. 494, which will be sent free from the Department, Washington, D. C.

SOMETHING ABOUT CALCIUM IN THE BODY.*

W. P. WHEELER.

Need for calcium in animal body. The prescence of " mineral " elements in the animal body has long been known, and the essential character of some of them, as well as the necessity for an adequate supply, recognized, but modern studies in animal nutrition have related chiefly to the energy and protein requirements as supplied by the organic constituents of the food. In recent years, however, much has been added to our knowledge of important rôles of the mineral elements, altho there remains very much to learn concerning some of their relations.

From the feeders' standpoint one of the most important elements to consider is calcium. Of the mineral elements serving in the bodies of all farm animals (as well as of man) this one is found in largest amount. Many foods contain only very small amounts of calcium, and rations which consist largely of such foods, as they often do under modern conditions, fail to supply enough of this element.

Much calcium is required by the animal while its bones are hardening and by those mammals giving milk. During extended egg production by birds a large amount is needed. The obvious and somewhat exceptional demands for calcium by domesticated fowls while laying makes them good subjects for certain studies relating to its metabolism.

Experiments with poultry. The special physiological activities observed for one class of animals cannot be attributable in all particulars without qualification to another class. For instance, we find some difference in the response to unusual shiftings of the nutrients in the ration even between representative species of two classes of birds that under domestication have long been grown and maintained on substantially identical foods.

* Reprint of Popular Bulletin No. 468, December, 1919. The complete bulletin is reprinted on p. 55.

All the data we have, however, indicate that in a general way a knowledge of the influence of certain factors profoundly affecting the life of one warm blooded vertebrate may justifiably be used to help to an understanding of the needs of certain others.

The results of some experiments reported in Bulletin No. 468, while more directly relating to poultry feeding, may be of some general interest at this time when more attention is being given than formerly to the importance of considering the calcium content of food for man as well as for certain farm animals. Because it was not possible at any one time to give sufficient attention to this special line of work, the different feeding trials in these experiments were scattered over a number of years at irregular intervals, most of them having been made a number of years ago.

Magnesium not a substitute for calcium.

Besides the special duties of the base-forming elements in the body, they all serve their part in maintaining neutrality wherever necessary. In this direction, magnesium, which is nearest to calcium chemically of any recognized element in the body or its food, may work with calcium to a certain extent, altho in some important functions their action is antagonistic. But in the bony framework, in so far as it serves for mechanical support, and in the shells of birds' eggs, both structures having the two elements as normal constituents, it would appear that magnesium might serve to a limited degree in place of calcium. That it does this, however, to an extent that can be considered important or more than incidental or accidental, we have failed to find.

On the other hand, strontium, nearer to calcium chemically than other elements, can replace calcium to a certain extent, altho it is not a recognized normal constituent of the body nor of ordinary foods.

General plan of experiments.

When first attempting to trace the transference of lime from the food to the egg-shell, it seemed that, if the relative proportion deposited in the shell should vary markedly and in the same direction as in different sources of supply, there would be presumptive evidence that the material came directly or indirectly from the fluctuating source. There is a small but reasonably constant percentage of magnesium in the shell. It was a question whether this base-forming element would replace calcium to any extent when salts of magnesium were supplied with food deficient in calcium.

Because strontium is seldom found in any appreciable amount in food and ordinary water, because it approaches calcium in chemical properties, and because the ordinary salts of strontium are not poisonous, this element seemed especially adapted for use in such an investigation. Strontium salts were, therefore, used in certain rations.

At first several attempts were made to provide a basal ration largely of food substances so purified as to contain either little or practically none of certain ash constituents, the amount desired being

supplied as added salts. But no satisfactory results were secured with any ration that did not contain considerable food in its natural, or not specially purified, state. Several rations composed largely of prepared foods served for maintenance for quite long periods without apparent harm to mature birds, but would not sustain or induce reproduction. There was no opportunity to prepare and try out any considerable assortment of the possible purified products, and there were failures to secure eggs either at all or in significant numbers from rations containing a desired proportion of the products available. So it was necessary to use " compromise " rations with as small a proportion of the needed natural foods as would assure a fair growth of young, and eggs in significant numbers from the mature birds. There was in every basal ration an inadequate supply of calcium, however, and, as is the case with practically all rations consisting largely of grain and meat foods that do not include bone, from three to four times as much magnesium as calcium.

Selective material in the egg-shell

In one of the earlier feeding trials there was observed a failure of magnesium to replace calcium appreciably in the egg-shell under conditions where strontium did replace calcium to a considerable extent. In this trial four lots of hens were fed an ordinary ration somewhat low in calcium content as usual, and were in addition supplied, one lot with oyster-shell, another with magnesite, another with strontianite, and a fourth with hard glass. The percentage of calcium in the egg-shells remained very constant for each lot except the lot fed strontianite, in which case about one-fourth of the calcium was replaced by strontium. The slight variations in the percentage of magnesium did not suggest any appreciable replacement of calcium by this element when magnesite was fed.

In a later feeding trial with young growing pullets, on rations deficient in calcium and with either magnesium or calcium added in the form of pure salts, the first eggs laid as the birds approached maturity showed only slight differences in the composition of the shells.

With young hens fed rations similarly deficient in calcium, in another feeding trial, there was the same failure of magnesium to replace calcium in the egg-shell. Shells of eggs from hens fed magnesium carbonate carried a slightly smaller percentage of magnesium on the average than was in shells of eggs from hens fed either ground oyster-shell or strontium carbonate. But strontium did replace calcium to a large extent in the egg-shell when strontium carbonate was fed.

The same general distribution of the principal elements in the egg-shell was found when three other lots of hens were fed rations deficient in calcium as usual with different salts added as desired. With practically the same amount of shell on the egg there was slightly

more magnesium and slightly less calcium from hens fed magnesium salts (mixed carbonate and phosphate) than from hens fed added calcium (carbonate and phosphate). The variations in composition as between the two lots were no greater than occur under identical rations. But again strontium did replace calcium in the shell to a large extent. The average from a few eggs of thin shells after three months' feeding showed even more strontium than calcium.

In another feeding trial, eggs from ducks fed magnesium carbonate with the usual calcium-deficient ration had shells of practically the same composition as those from ducks fed calcium carbonate. Eggs from ducks fed strontium carbonate with the same ration showed a slight replacement by strontium even on the second day of feeding.

When three other lots of ducks were fed a basal ration low in calcium with either magnesium, calcium, or strontium added as carbonates and phosphates, the shells of eggs from the birds fed magnesium salts had nearly the same composition as those from others fed calcium salts. Shells of eggs (altho few) from the ducks fed strontium salts contained a little less magnesium than the others, and strontium in place of part of the calcium.

Calcium replacement in the bones.

If calcium is used in any considerable amount above that in the food and water, for essential physiological functions where no substitute will serve and for such structures as the egg-shell, it must come from what is stored in the body. The greater part of this reservoir of calcium is in the bones. From eight to nine percent of the total dry matter of the body of an average hen is represented by the ash constituents. A large part of this ash is calcium phosphate.

It was assumed that if calcium were withdrawn from the bones it would be taken first and perhaps to a larger extent from the softer bones having more blood vessels. The results with the common fowl were usually in accord with this assumption when mature or nearly grown young birds were used. The rule did not always hold in the earlier stages of growth when the bones had not yet fully ossified, and when there was perhaps less difference between them.

The effect of the deficient rations on the softer bones was much more noticeable in their physical appearance than in the figures from chemical analysis. Under some rations, the breast bone and the bones of the pelvic arch, especially, lost all stiffness, became soft, flexible and translucent, and apparently without much lime, altho analysis showed considerable of the mineral matter still remaining.

With the duck, young and old, a fowl which seems better able to adapt itself to an excess of magnesium when only a very low indispensable minimum of calcium is present, the relations between the two classes of bones in respect to changes in composition did not hold always as with the common fowl.

Where strontium replaced calcium in the bones, however, in every instance with both representative species, except in the earlier stages of feeding with immature birds, the ratio of strontium replacement was higher in the softer bones.

Presence of strontium in the body.

If strontium were transported freely thru the body either in addition to calcium or partly as a substitute, we should expect to find it wherever its presence did not vitally interfere with the special functions of other elements.

The relative proportion of strontium to calcium found in the egg itself was not much different from that existing in the shell, and this proportion was about the same in the white as in the yolk. Usually, but not always, both in the white and in the yolk, there was less than the usual amount of calcium whenever strontium was present. The absolute amounts of these elements in the egg are small, however, and not much importance can be attached to variations in the relative amounts in the absence of sufficient data to show what may be an ordinary range of the variation in composition that has been observed under normal rations.

Strontium was found in the muscles in smaller amounts in relation to calcium than in the egg, altho the proportion was higher in the "red" muscles than in the "white" muscles.

Except in a few egg-shells, the only place where strontium was found in considerably greater amount than calcium was in the smaller feathers from a hen that had been fed strontium salts for more than one year, having been carried thru a full molt. In these smaller feathers there was much more strontium than calcium, altho in the larger feathers there was a little more of calcium than of strontium. A difference of the same kind was found in feathers from a yearling duck fed strontium salts, after reaching full size, for a period of six months not covering any general molt. In this instance there was found half as much strontium as calcium in the smaller feathers, and about one-fifth as much in the larger feathers.

General differences in bone material.

Whenever the old or young of the common fowl or duck were fed rations low in calcium content with added magnesium salts, there was soon found in the bones less calcium and less total mineral matter than in bones from birds fed the same ration with added calcium salts.

Usually, but not always, those birds fed rations with added magnesium salts had more magnesium in the bones than the birds fed calcium salts, altho the actual difference in amount of magnesium was always small. Except with young ducks or very immature chicks, birds fed strontium salts for any considerable time had more magnesium in the bones than those fed magnesium or calcium salts for the same time.

When strontium salts were fed for several weeks or several months with these low-calcium rations, mature hens or nearly mature chicks always had heavier bones with more mineral matter in them, actually and in relation to body weight, than did similar birds fed corresponding calcium or magnesium salts.

There was a similar result when mature ducks were fed such rations for a limited time, but not with immature ducks or young ducklings, or very immature chicks.

Tolerance for the unusual rations. Under these unusual, and somewhat artificial, rations the common fowl on the whole was much better able to endure the feeding of strontium salts than was the duck. With variety provided in the food and no extreme deficiency of calcium, mature hens could be fed the added strontium for a long time without any noticeable injury that could be attributed to the presence of this element in the food.

Mature ducks of large size did not suffer any apparent harm during limited periods of feeding from the presence of strontium in the food. But under such rations as were used, immature ducks or growing ducklings had little tolerance for strontium carbonate or phosphate never being in best condition and making very slow growth. On the other hand, altho there was a shortage of calcium in the bones and a failure of magnesium to replace it, rations with added magnesium salts were practically equal in efficiency to those with added calcium salts and permitted as rapid, and, except as to bone development, apparently as normal growth. The small amount of calcium in the food, supplemented by any temporarily withdrawn from the bones, apparently provided the indispensable minimum and there seemed almost perfect tolerance for the added magnesium salts, so that in general appearance and condition of plumage the young ducks often surpassed those fed calcium salts.

With the ducklings started on these rations at the earlier age and fed for the longer periods, there was, on the average for the whole time, no difference in the amount of dry matter of the food required per pound increase in weight between those fed added magnesium and those fed added calcium. For the common chick, up to the time when nearly half grown, the ration with added magnesium permitted fully as rapid growth from the same amount of dry matter in the food as the ration with added calcium, but from this stage of growth on the efficiency of the rations with added calcium salts was increasingly the greater.

Fat in the body. The ability to save or to increase the stores of fat in the body under the unusual rations, so far as indicated by ordinary dissection and, in several instances, by analyses of the bones, appeared to be considerably different for the two representative species.

For the common fowl there was in every trial, after several months feeding, more fat next the skin and about the body with the birds fed the added calcium salts. Between those fed the magnesium or strontium salts there was not much difference on the average. The feeding of strontium salts with a varied but low-calcium ration for over a year to one hen did not prevent an increase in this proportion of stored fat. Young pullets approaching laying maturity, after they had been under these rations for six to eight months, showed a larger percentage of fat in the trunk bones from those fed the added magnesium than from the others, and there was considerable shortage of fat in the harder bones from those having had strontium salts. Elsewhere in the body, however, there was more fat with the lot fed added calcium salts than with the other lots between which there was little difference.

The duck, young or old, when fed the ration with added strontium salts for any considerable time showed a noticeable lack of fat in the bones and in the rest of the body. A partial exception was in the case of a laying duck fed for six months on the restricted ration when the hard bones showed no apparent loss of fat, altho there was a noticeable shortage in the softer bones and elsewhere in the body. There were abundant stores of fat in the bones and elsewhere with birds fed the added magnesium and calcium salts. On the whole, for the longer periods of feeding with the ducks, slightly more fat was held or accumulated under the ration with added magnesium than under the rations with added calcium salts.

Effects on egg production.

In the matter of influence on egg production, the results, on the whole, plainly favored the rations with the larger proportion of calcium. Under the unusual rations free laying could not be expected, but three and four times as many eggs were obtained from hens fed the added calcium as from the other lots. Fewer eggs were obtained from the hens fed added magnesium salts than from the hens fed strontium salts. With the younger hens from which readier egg production would normally be expected, laying started earlier and continued longer with the lot fed added calcium salts.

Few eggs were obtained from ducks under such rations, but on the average there were a few more from those fed rations with the added calcium, altho in one trial as many eggs were obtained from the ducks fed added magnesium salts. Only very few eggs were ever obtained from ducks fed strontium salts for any considerable time.

Ordinary food-calcium inadequate.

The average results from several feeding trials with hens and ducks and with chicks and ducklings are reported in the "complete edition" bulletin in more or less detail. At many times, in several feeding trials, it was observed that the amount of calcium in the food for certain periods fell very far short of the amount in the eggs.

With one lot of hens fed magnesite with the basal ration the shells of the first few eggs laid contained fifteen times as much calcium as was in the food and drinking water for the period during which these eggs were produced. With another lot fed magnesium salts the total calcium in the food and water for one period of six weeks was less than seven percent of the amount of calcium in the shells of eggs laid during that time.

Shells of the first few eggs laid by one lot of hens fed strontium carbonate contained over eight times as much calcium as was in the food and water during the period of their production. During one period of about three weeks with another lot fed strontium salts the shells of eggs produced carried three and one-half times as much calcium as was in the food and water and about fifteen percent more of strontium than of calcium.

During each of two periods, one of four and one of five weeks, there was in the food, aside from the added calcium salts fed to one lot of hens, less than four percent of the amount of calcium in the eggs produced. For a total of six months with another lot of hens the calcium in all the food (including water), aside from the added oyster-shell, was 4.2 percent of the amount in the shells of eggs laid during the same period.

It was concluded that, under the rations fed, the larger part of the calcium and of the total mineral matter used for egg production came sometimes from the bones and sometimes directly and indirectly from the mineral salts fed.

Conclusion. Calcium and magnesium are quite generally and abundantly distributed by nature. Many hills and mountains consist in part or almost wholly of compounds of these two elements in close association but varying proportion. They exist together in most soils and in plants. In the animal body they are found in abundance. Altho in the soft tissues of the body certain functions of these elements are somewhat opposite, they work together in nature in many ways. But all the data available indicate that, for certain apparently simple associated service in the body, one does not take the place of the other.

In the usual food for the hen, as ordinarily managed under modern conditions, there is considerably more magnesium than calcium, altho more calcium is required by the body. At times much more calcium is needed than is supplied by the food, and must be withdrawn from the reservoir in the bones. The skeleton is more than a foundation support of inert substance. This structure, so rich in mineral matter, is not by any means composed of dead material, but is subject to growth and depletion as are the softer tissues.

Results in experiments, started a number of years ago but not completed, are not out of accord with the idea that both calcium and phosphorus are more easily taken from the bones than from dead bone ash in the food. Even with the cow, an animal especially

adapted to use in bulk the coarser foods richer in lime, there is often a negative balance of calcium during most of the milking period, altho her food may be enriched by added calcium compounds.

Most grain foods and some other foods are deficient in calcium, and neither the hen nor duck can consume enough of the bulky foods to meet the calcium requirement of the laying period. The mature fowl can, however, use the calcium in inorganic food materials without much apparent disadvantage. The very young fowl cannot, with advantage, so freely supply its needs from these inorganic materials in the same form. For this, as well as for other reasons, it is important to provide a regular supply of the vegetable foods, richer in calcium, in their best condition. While it is very unlikely, under any normal conditions, that the animal will lack the small amounts of calcium required to insure proper heart action and maintain essential properties of the blood, and for other such rôles where calcium alone will serve, the store may become too small to promptly meet certain exceptional demands without harmful stress. It is not, therefore, the part of wisdom to permit this very important reservoir of mineral elements to be depleted much, especially during growth, or except in the emergencies for which it is needed and provided.

PERIODICALS RECEIVED BY THE STATION.

Abstracts of Bacteriology	Subscription
Agricultural Gazette of Canada	Complimentary
Agricultural Gazette of New South Wales	Complimentary
Agricultural Journal of India	Complimentary
Agricultural Review	Subscription
American Agriculturist	Subscription
American Chemical Society Journal	Subscription
American Entomological Society (Transactions)	Subscription
American Fertilizer	Subscription
American Florist	Subscription
American Fruit Grower	Subscription
American Grocer	Complimentary
American Journal of Botany	Subscription
American Journal of Physiology	Subscription
American Journal of Science	Subscription
American Museum of Natural History, Bulletin	Complimentary
American Naturalist	Subscription
American Philosophical Society, Proceedings	Subscription
American Poultry Advocate	Complimentary
American Seedsman	Exchange
American Society of Agronomy, Journal	Subscription
Analyst	Subscription
Annales de L'Institut Pasteur	Subscription
Annali della R. Scuola sup. d'Agriculture, Portici	Complimentary
Annals and Magazine of Natural History	Subscription
Annals of Applied Biology	Subscription
Annals of Botany	Subscription
Annals of the Missouri Botanical Garden	Subscription
Archiv. für Hygiene	Subscription
Archiv. für Physiologie	Subscription
Berichte der deutschen chemischen Gesellschaft	Subscription
Better Fruit	Subscription
Biochemische Zeitschrift	Subscription
Biological Bulletin	Subscription
Biologisches Centralblatt	Subscription
Boletim de Agricultura	Complimentary
Boletin de la Sociedad Entomologica de Espana	Complimentary

Botanical Abstracts	Subscription
Botanical Gazette	Subscription
Bulletin tri. Société Mycologique	Subscription
California Academy of Sciences, Proceedings	Complimentary
California Cultivator	Subscription
California Fruit News	Subscription
California University Publications—Agricultural Sciences, Botany and Zoology	Complimentary
Canadian Entomologist	Subscription
Canadian Horticulturist	Complimentary
Centralblatt für Bakteriologie, Parasitenkunde und Infektions krankheiten	Subscription
Chemical Abstracts	Subscription
Chemical Society, Journal	Subscription
Chemisches Zentralblatt	Subscription
Chicago Dairy Produce	Subscription
Climatological Data	Complimentary
Commercial Fertilizer	Subscription
Congressional Record	Complimentary
Country Gentleman	Subscription
Country Life	Subscription
Creamery and Milk Plant Monthly	Subscription
Curtis' Botanical Magazine	Subscription
Dairy Farmer	Subscription
Duroc Bulletin	Complimentary
Entomological News	Subscription
Entomological Society of America, Annals	Subscription
Entomological Society of Washington, Proceedings	Subscription
Entomologist	Subscription
Entomologist's Record	Subscription
Experiment Station Record	Complimentary
Farm Journal	Complimentary
Farmers' Advocate	Complimentary
Feathered World	Subscription
Florists' Exchange	Subscription
Flour and Feed	Subscription
Garden	Subscription
Gardeners' Chronicle	Subscription
Garden Magazine	Subscription
Gartenwelt	Subscription
Gleanings in Bee Culture	Subscription

Hawaiian Forester and Agriculturist	Complimentary
Hoard's Dairyman	Subscription
Hospodar	Complimentary
Insect World (Japanese)	Complimentary
Internationale Mitteilungen für Bodenkunde	Subscription
International Garden Club, Journal	Subscription
Journal of Agriculture, New Zealand	Complimentary
Journal of Agricultural Research	Complimentary
Journal of Agricultural Science	Subscription
Journal of American Medical Association	Subscription
Journal of the American Peat Society	Subscription
Journal of Association of Official Agricultural Chemists	Subscription
Journal of Bacteriology	Subscription
Journal of Biological Chemistry	Subscription
Journal of Board of Agriculture (English)	Complimentary
Journal of the College of Agriculture, Tokyo	Complimentary
Journal of Dairy Science	Subscription
Journal of the Department of Agriculture of South Australia	Complimentary
Journal of the Department of Agriculture of Victoria	Complimentary
Journal of Experimental Medicine	Subscription
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Journal of Industrial and Engineering Chemistry	Subscription
Journal of Pathology and Bacteriology	Subscription
Journal of Physical Chemistry	Subscription
Journal of Physiology	Subscription
Landwirtschaftliche Jahrbuch der Schweiz	Subscription
Landwirtschaftliche Jahrbücher	Subscription
Landwirtschaftliche Versuche Stationem	Subscription
Leghorn World	Complimentary
Market Growers' Journal	Complimentary
Market Reporter	Complimentary
Memoirs of the Department of Agriculture in India	Complimentary
Michigan Farmer	Complimentary
Milchwirtschaftlisches Zentralblatt	Subscription
Monthly Bulletin, International Institute of Agriculture	Complimentary
Monthly Bulletin of the N. Y. State Department of Health	Complimentary
Monthly Crop Reporter	Complimentary

Monthly Weather Review	Complimentary
Mycologia	Subscription
National Farmer	Complimentary
National Nurseryman	Subscription
National Weather and Crop Bulletin	Complimentary
Naturaliste Canadienne	Complimentary
New York Academy of Science, Annals and Transactions	Subscription
New York Botanical Garden, Bulletin	Complimentary
New York Entomological Society, Journal	Subscription
New York Produce Review	Subscription
New York State Museum Bulletins	Complimentary
Northwestern Miller	Complimentary
Ohio Farmer	Complimentary
Ohio Journal of Science	Subscription
Pacific Dairy Review	Complimentary
Pacific Poultry Breeder and Fanciers' Monthly	Subscription
Parasitology	Subscription
Photo-Miniature	Subscription
Phytopathology	Subscription
Plant World	Subscription
Potato Magazine	Subscription
Poultry Herald	Subscription
Poultry Item	Complimentary
Power	Subscription
Proceedings Boston Society of Natural History	Complimentary
Psyche	Subscription
Reclamation Record	Complimentary
Reliable Poultry Journal	Subscription
Review of Applied Entomology	Subscription
Revue de Viticulture	Subscription
Revue Générale de Botanique	Subscription
Revue Horticole	Subscription
Royal Agricultural Society Journal	Subscription
Rural New Yorker	Subscription
Science	Subscription
Scientific American	Subscription
Seed World	Subscription
Société Entomologique de France, Bulletin	Complimentary
Soil Science	Subscription
Stazione Sperimentale Agrarie Italiane	Complimentary
Torrey Botanical Club, Bulletins and Memoirs	Subscription

Wallace's Farmer	Complimentary
Wilson Bulletin	Complimentary
Zeitschrift für analytische Chemie	Subscription
Zeitschrift für Biologie	Subscription
Zeitschrift für Botanik	Subscription
Zeitschrift für Pflanzenkrankheiten	Subscription
Zeitschrift wissenschaftliche Insektenbiologie	Subscription
Zoölogische Anzeiger	Subscription

METEOROLOGICAL RECORDS FOR 1919.

METEOROLOGICAL RECORDS FOR 1919.

READING OF STANDARD AIR THERMOMETER.

Date	January			February			March			April			May			June		
	7 A. M.	12 M.	5 P. M.	7 A. M.	12 M.	5 P. M.	7 A. M.	12 M.	5 P. M.	7 A. M.	12 M.	5 P. M.	7 A. M.	12 M.	5 P. M.	7 A. M.	12 M.	5 P. M.
1	38	47	49	16	19	23	30	29	29	15	20	19	45	50	49	63	82	85
2	31	30	28	17	28	30	23	44	40	21	32	38	49	50	54	65	86	87
3	25	25	20	18	34	37	28	44	43	38	52	45	40	54	53	68	85	95
4	9	15	14	37	40	38	38	58	58	38	45	48	52	65	64	74	88	92
5	8	23	10	22	22	24	35	30	22	43	57	56	56	53	53	73	85	84
6	4	20	17	20	28	24	14	20	19	43	61	54	43	56	64	68	79	70
7	22	30	30	20	30	30	10	28	24	50	64	55	43	61	70	67	82	76
8	31	35	32	24	29	25	14	30	35	36	50	45	46	54	52	66	74	70
9	30	30	15	21	27	23	36	40	45	37	45	49	43	46	50	62	66	65
10	5	22	30	15	21	20	31	40	42	44	47	53	45	44	42	64	78	79
11	15	10	6	8	29	27	35	36	38	54	45	45	40	43	47	65	80	79
12	1	18	18	25	34	35	30	43	50	44	46	47	42	46	49	64	80	81
13	22	30	37	28	37	38	30	18	17	39	42	44	54	68	71	65	82	81
14	40	43	35	38	39	43	13	27	33	39	50	50	54	75	71	70	87	90
15	30	35	30	35	39	33	28	38	35	35	44	41	56	67	60	77	87	85
16	32	45	39	20	19	26	34	38	40	43	43	44	54	65	61	71	86	74
17	40	45	47	23	30	29	45	54	55	43	47	44	53	58	55	68	84	85
18	30	48	44	23	28	26	39	37	37	40	43	44	49	60	59	73	86	87
19	34	34	30	17	22	23	31	34	30	36	53	53	55	65	63	70	86	84
20	25	40	43	17	34	30	33	50	49	45	59	50	50	57	61	73	75	77
21	27	45	47	20	32	33	38	45	40	36	44	48	57	67	66	69	75	74
22	40	48	45	32	37	37	25	28	30	36	56	64	61	66	57	63	70	71
23	38	42	42	35	38	36	32	43	44	45	68	70	57	65	54	64	80	85
24	30	23	23	31	36	38	32	44	42	48	37	30	55	57	55	69	85	88
25	21	31	32	32	38	37	31	48	54	22	27	25	52	67	68	69	69	66
26	36	37	35	19	20	18	40	59	64	28	35	39	60	76	78	67	77	78
27	31	35	33	15	26	30	51	53	31	43	55	60	62	69	63	65	59	64
28	26	40	38	31	50	44	18	22	28	48	49	47	54	74	72	54	60	60
29	28	31	30	....	....	....	22	25	26	36	45	49	58	78	82	54	67	70
30	28	35	37	....	....	....	25	30	31	43	51	56	67	82	79	56	78	83
31	27	26	25	....	....	....	24	20	18	....	....	....	63	83	85	....	....	....
Averages	25.9	32.9	31.0	23.9	30.9	30.6	29.5	37.2	37.0	38.9	41.0	46.7	52.9	62.9	62.4	66.5	78.6	78.8

READING OF STANDARD AIR THERMOMETER (*concluded*).

Date	July			August			September			October			November			December		
	7 A. M.	12 M.	5 P. M.	7 A. M.	12 M.	5 P. M.	7 A. M.	12 M.	5 P. M.	7 A. M.	12 M.	5 P. M.	7 A. M.	12 M.	5 P. M.	7 A. M.	12 M.	5 P. M.
1	66	85	87	63	68	69	60	70	68	46	60	63	59	52	46	22	27	28
2	69	88	90	63	71	72	65	67	68	55	70	84	39	40	39	26	31	25
3	70	91	92	65	74	74	60	75	75	70	80	76	28	40	45	11	20	16
4	77	94	94	63	83	84	61	75	73	61	85	83	47	47	44	14	28	24
5	77	84	93	67	73	71	52	70	72	67	80	75	35	43	40	24	31	30
6	65	66	63	69	77	81	59	83	82	68	67	65	36	40	38	17	30	37
7	65	70	73	70	86	92	74	90	87	56	53	48	34	37	35	34	36	33
8	61	74	77	59	64	72	77	90	90	33	53	55	35	40	35	35	42	35
9	65	80	79	56	67	70	63	79	69	50	54	57	28	35	37	38	39	44
10	65	78	66	56	70	77	62	67	62	60	79	77	35	48	50	30	25	19
11	51	64	68	51	78	76	62	72	70	51	47	47	47	57	49	12	21	22
12	58	73	72	65	77	80	55	59	59	35	47	49	47	58	56	33	39	40
13	62	80	81	65	76	79	53	65	70	38	57	60	29	35	31	46	44	35
14	67	83	85	63	75	74	59	72	73	47	57	58	29	37	40	25	24	18
15	75	71	77	64	79	76	55	64	66	55	69	72	27	36	34	15	17	10
16	62	67	72	64	84	82	56	71	67	54	57	50	30	49	44	5	11	10
17	65	78	83	65	66	68	57	62	60	45	51	51	40	50	51	5	3	—2
18	69	84	84	65	74	71	44	66	72	39	55	56	45	50	40	0	13	6
19	71	84	86	65	74	75	58	67	65	45	55	50	24	28	25	8	12	10
20	70	77	82	64	75	81	68	81	83	37	45	46	19	24	27	7	22	23
21	70	80	78	64	77	76	69	79	75	38	55	55	28	47	47	24	30	29
22	74	85	83	66	80	81	70	79	65	47	54	54	43	47	43	20	33	30
23	68	79	82	63	80	82	53	57	59	40	55	56	38	43	34	23	36	35
24	72	85	84	78	80	77	55	68	65	45	57	60	32	38	35	30	24	19
25	66	74	80	60	66	58	59	61	61	52	59	59	34	39	41	13	16	13
26	65	73	83	56	63	60	49	60	57	60	62	54	33	32	30	23	34	34
27	78	88	89	51	62	64	42	61	70	44	46	46	27	28	26	34	35	35
28	74	81	83	56	63	68	52	74	79	64	56	45	20	26	28	22	22	15
29	65	74	72	55	73	79	59	80	75	36	46	42	37	42	45	5	20	18
30	65	74	77	63	69	72	60	52	50	43	47	50	39	35	30	22	19	27
31	65	70	66	59	66	68	....	....	....	57	71	67	....	....	....	18	28	31
Averages	67.5	78.5	80.0	62.3	73.2	74.4	55.6	70.5	69.5	49.6	59.0	58.7	34.7	40.8	38.8	20.5	20.5	23.8

READING OF MAXIMUM AND MINIMUM THERMOMETERS FOR 1919.

Date	January 5 P. M. Max.	January 5 P. M. Min.	February 5 P. M. Max.	February 5 P. M. Min.	March 5 P. M. Max.	March 5 P. M. Min.	April 5 P. M. Max.	April 5 P. M. Min.	May 5 P. M. Max.	May 5 P. M. Min.	June 5 P. M. Max.	June 5 P. M. Min.
1	50	31	32	16	44	27	24	10	56	42	87	56
2	49	29	34	17	45	23	39	18	60	44	92	59
3	30	20	40	17	50	22	53	30	63	39	96	60
4	21	7	42	27	65	29	53	37	69	44	95	61
5	23	7	40	21	58	22	59	39	64	49	92	60
6	20	1	30	19	25	14	61	36	65	32	85	63
7	32	14	36	20	30	3	67	44	71	46	82	59
8	35	29	33	21	37	12	57	33	70	38	79	62
9	37	15	29	18	46	30	51	37	54	40	77	59
10	31	1	29	9	50	30	54	42	52	42	81	58
11	30	6	35	8	44	33	60	44	48	40	81	56
12	24	—1	37	25	53	27	49	41	50	41	84	53
13	38	16	40	23	50	17	47	39	74	43	85	60
14	45	34	47	36	34	12	54	33	78	42	92	64
15	37	30	45	32	40	27	52	30	71	43	90	66
16	48	28	33	19	42	32	46	33	68	46	89	64
17	47	38	35	22	61	33	49	41	69	52	86	66
18	54	29	31	22	55	37	48	36	62	44	91	63
19	45	30	27	17	40	30	57	32	70	43	90	67
20	49	24	36	17	60	30	64	33	66	45	85	68
21	55	27	34	25	49	35	52	33	69	55	78	60
22	54	30	40	31	40	23	65	27	70	57	77	50
23	47	38	40	33	48	25	71	34	72	48	86	49
24	42	23	41	31	47	31	70	30	60	45	90	59
25	40	20	42	21	58	24	30	20	70	47	89	65
26	42	30	37	18	69	34	40	20	80	55	84	66
27	38	31	32	15	64	31	61	34	83	59	78	58
28	44	26	54	21	33	18	61	42	76	45	65	47
29	40	28	......	......	31	21	51	36	85	49	72	42
30	39	28	......	......	35	22	60	34	83	54	80	54
31	37	25	......	......	31	18	......	......	86	55	......	......
AVERAGES	39.5	22.4	36.8	21.5	36.6	25.0	53.5	33.6	68.1	45.9	83.9	59.1

READING OF MAXIMUM AND MINIMUM THERMOMETERS FOR 1919 (*concluded*).

DATE	JULY		AUGUST		SEPTEMBER		OCTOBER		NOVEMBER		DECEMBER	
	5 P. M. Max.	5 P. M. Min.	5 P. M. Max.	5 P. M. Min.	5 P. M. Max.	5 P. M. Min.	5 P. M. Max.	5 P. M. Min.	5 P. M. Max.	5 P. M. Min.	5 P. M. Max.	5 P. M. Min.
1	89	53	71	60	72	50	66	44	71	46	39	20
2	93	55	74	53	70	54	87	53	51	38	32	24
3	94	58	76	54	76	58	85	62	45	27	28	9
4	95	64	86	56	75	59	87	60	50	40	29	9
5	95	70	85	64	73	44	85	61	50	34	34	19
6	93	63	82	66	86	50	76	64	44	35	33	16
7	73	55	92	68	92	68	67	47	40	38	36	27
8	77	50	92	56	91	70	56	30	43	33	43	32
9	83	53	72	47	90	62	57	44	41	26	45	31
10	82	64	77	45	70	58	82	57	55	27	44	20
11	74	49	79	51	76	60	77	45	63	46	26	11
12	76	50	81	53	72	54	50	34	63	36	43	22
13	82	55	82	61	70	49	61	30	59	29	50	34
14	86	53	79	51	74	52	61	40	41	24	38	18
15	85	70	81	56	74	53	72	52	40	25	23	10
16	79	60	86	56	73	54	72	49	57	25	11	5
17	84	50	82	64	71	53	57	41	59	39	15	—2
18	85	56	75	68	72	39	59	34	52	43	15	—6
19	89	64	78	59	72	54	58	38	45	23	14	—2
20	87	67	81	56	85	61	55	34	45	18	24	2
21	84	69	81	60	86	64	56	33	49	24	34	21
22	87	68	82	59	80	64	57	46	50	40	37	19
23	83	62	84	56	66	51	59	36	45	33	41	14
24	86	60	84	68	70	49	65	41	42	30	36	19
25	84	59	78	56	73	51	65	51	42	31	19	12
26	83	61	66	52	63	40	65	55	44	30	35	6
27	90	61	66	49	71	37	59	43	36	25	37	33
28	90	70	70	50	81	38	70	45	31	19	35	14
29	83	61	79	49	81	53	46	35	47	28	24	3
30	78	58	79	57	80	49	56	32	45	30	27	12
31	79	53	74	58	......	......	79	50	......	......	33	16
AVERAGES	84.7	59.3	79.2	56.4	76.2	53.2	66.0	44.7	48.1	31.1	31.6	15.1

SUMMARY OF AVERAGES OF MAXIMUM, MINIMUM, AND STANDARD AIR THERMOMETERS FOR 1919.

	JAN.	FEB.	MAR.	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.
Maximum	30.5	36.8	36.6	53.5	68.1	83.9	84.7	79.2	76.2	66.0	48.1	31.6
Minimum	22.4	21.5	25.0	33.6	45.9	59.1	59.3	56.4	53.2	44.7	31.1	15.1
Standard 7 A. M.	25.9	23.9	29.5	38.9	52.9	66.5	67.5	62.3	55.6	49.6	34.7	20.5
Standard 12 M.	32.9	30.9	37.2	41.0	62.9	78.6	78.5	73.2	70.5	59.0	40.8	20.5
Standard 5 P. M.	31.0	30.6	37.0	46.7	63.4	78.8	80.0	74.4	69.5	58.7	38.8	23.8

MONTHLY MAXIMUM AND MINIMUM TEMPERATURES FROM 1883 TO 1919, INCLUSIVE.

(Highest and Lowest Record for Each Month in Heavy Type.)

YEAR	JANUARY				FEBRUARY				MARCH				APRIL			
	MAX.		MIN.		MAX.		MIN.		MAX.		MIN.		MAX.		MIN.	
	Date	Temp.	Date	Temp.	Date	Temp.	Date	Temp.	Date	Temp.	Date	Temp.	Date	Temp.	Date	Temp.
1883	18	44.	11	—9.	17	48.	24	—2.	19	61.	9	2.	16	75.	1	19.
1884	14	42.	26	—13.	7	55.	29	—3.	30	54.	1	—4.	28	74.	1	23.
1885	1	61.	29	—6.	10	38.	11	—11.5	28	48.	13	—11.	24	84.5	10	20.5
1886	5	52.5	18	—18.7	9	50.	27	—11.	16	58.	2	—2.5	24	80.5	4	22.
1887	24	50.7	19	—8.	9	54.2	27	—4.	3	51.7	1 & 5	8.7	11	75.7	1	17.2
1888	2	43.2	23	—6.	21	49.	10	—7.	28	57.	13	0.	29	82.5	8	19.
1889	18	55.	20	5.	23	42.	4 & 24	—7.	28	61.8	30	18.5	20	84.	1	26.
1890	6	67.	29	9.	5	64.5	11&21	9.5	13	62.	8	2.	13	78.8	1& 19	23.
1891	3	46.	17	4.	26	56.8	15	2.5	12	57.	2	4.5	28	81.4	7	21.
1892	3	48.	10	—5.	15	44.	6	2.8	27	52.2	4	6.	6	78.	17	25.5
1893	29	46.	11	—6.	15	47.4	5	—1.	24	54.	5	9.	13	75.3	26.2	25.
1894	5	59.	13	11.	20	47.6	27	—8.5	18	73.	26	15.	21	71.3	2	20.
1895*	7	45.	19	4.	25	46.	8	—14.	25	52.	5 & 16	12.	30	80.	3	28.
1896	30	44.	6	—16.5	29	49.	17	—21.	31	56.5	24	—2.	17	87.	4& 5	19.
1897	5	58.	20	—3.5	18	49.5	1 & 27	5.5	21	64.	1	—1.	26	82.	20	19.
1898	13	57.	30&31	—4.	12	56.5	2 & 3	—2.	11	†65.	2	17.5	14&18	69.	5	18.
1899	5	59.	12	—4.	21	52.5	11	—8.	73	63.	21	13.	30	82.	3	23.
1900	23	56.	1	2.	14	57.	27	0.	10	46.	12	—3.	30	‡73.5	9	22.
1901	16	48.	20	—2.	16	36.	24	—2.5	24	67.	6	—1.	28	78.	12	28.
1902	3	44.	28	2.	28	52.	6	—3.	12	66.5	19	14.	22	87.	5	25.
1903	3	48.	9	—2.	28	62.5	18	—4.	19	78.5	1 & 2	19.	30	86.	5	21.
1904	23	48.	19	—14.	7	58.	16	18.	26	58.	4	8.	24	67.5	14	16.
1905	1	49.	26	—2.	20	45.	5 & 14	—6.	29	82.	5	1.	27&28	75.	16	23.
1906	21	71.	9	4.	24	64.	6 & 7	—7.	27	51.	25	2.	19	74.	2	25.
1907	6	53.	24	—18.	2	47.	12	—4.	29	83.	7	—1.	29	73.	2	19.
1908	22	45.	31	—9.	15	53.	2 & 5	—14.	28	73.	1	8.	27	78.	4	18.
1909	24	64.	19	—7.	5	52.	1	—1.	10	52.	5	5.	19	75.	11	12.
1910	2 & 22	45.	5	—8.	16	29.	7	—8.	24	82.	14&18	17.	4	84.	7	27.
1911	27&30	48.	5	—1.	17	52.	22	4.	27	60.	16	—1.	29	80.	8	18.
1912	18	44.	14	—12.	24	48.	10	—10.	31	62.	4	—1.	6	78.	4	19.
1913	17	57.	13	8.	20	65.	10	—10.	14&15	69.	7 & 8	1.	24	84.	20	24.
1914	29	51.	13&14	—9.	3	52.	13&24	—14.	26	65.	12	7.	19	82.	9 & 13	22.
1915	7	46.	30	—3.	21&23	53.	10	10.	25	50.	1	11.	25&26	88.	5	23.
1916	27	61.	17	—3.	1	47.	15	—8.	31	58.	18	—3.	21	73.	8	26.
1917	6	47.	11&12	1.	26	55.	13	—8.	31	68.	6 & 29	11.	22	80.	11&18	23.
1918	12	40.	2	—10.	20	55.	5	—11.	20	74.	11	7.	1&29	73.	9	23.
1919	21	55.	12	—1.	28	54.	11	8.	26	69.	7	3.	28	71.	1	10.

* Data from record kept by Mr. Edgar Parker for the year 1895; Station record not available.
† Maximum for first eleven days only. Record incomplete.
‡ Thermometers broken. Record not taken from April 19th to 24th, inclusive.

MONTHLY MAXIMUM AND MINIMUM TEMPERATURES FROM 1883 TO 1919, INCLUSIVE (*continued*).

(Highest and Lowest Record for Each Month in Heavy Type.)

YEAR	May Max. Date	May Max. Temp.	May Min. Date	May Min. Temp.	June Max. Date	June Max. Temp.	June Min. Date	June Min. Temp.	July Max. Date	July Max. Temp.	July Min. Date	July Min. Temp.	August Max. Date	August Max. Temp.	August Min. Date	August Min. Temp.
1883	11	87.	1 & 14	31.	7	86.5	2	42.	5	89.	1	46.	23	92.	15	46.
1884	24	88.	30	32.	25	90.	15	41.	2	87.5	15	50.	20	95.	25	44.
1885	18	81.7	3	27.5	14	86.5	23	41.5	18	90.5	12	46.5	1	89.	28	45.
1886	23	79.5	17&18	37.2	14	86.2	1	42.2	7	95.	1	45.2	30	91.5	6	47.7
1887	23	88.2	14	37.5	17	89.2	15	47.7	3	95.5	11	58.7	8	88.5	8	46.
1888	13	79.8	3	29.	23	94.1	4	40.	5	89.8	16	47.	9	92.6	23	48.3
1889	18	91.8	29	32.	22	85.	5	46.	11	90.7	6	50.5	31	86.7	16&17	50.3
1890	4	80.7	2	30.	30	85.6	8	44.8	9	94.5	24	46.5	4	96.2	24	46.0
1891	11	85.5	4	29.5	16	95.	6	40.	14	92.	31	46.	12	92.	29	46.5
1892	31	78.	9	34.2	14	92.	11	45.8	29	96.3	2	46.4	10	95.5	28	49.
1893	25	88.	9	35.	21	94.	1	44.	26	95.5	24	48.4	11	94.5	13	49.
1894	2	85.4	14	32.6	23	91.6	6	39.	21	97.	10	49.6	25	98.	27	45.3
1895*	31	92.	13&21	36.	3	**96.**	7	54.	8	94.	11	52.	11	88.	22	44.
1896	11	87.5	7 & 20	40.	21	89.	3	41.	3	94.	18	49.	6 & 7	96.	20	44.
1897	24	80.	8	32.5	24&25	87.5	2	42.	11	97.	15	57.	15	87.5	21	46.
1898	29	79.	6	34.	9	90.	16	40.	4	96.5	12	40.	24	90.5	28	47.
1899	2	87.5	15	32.5	6 & 24	93.	11	41.5	4	97.5	1	50.	20	97.5	15	44.5
1900	15&16	88.5	7	27.	25	93.	10	45.	17	96.	1	50.	11	97.	2	51.
1901	23	78.	16	36.	27, 28 & 29	95.5	2	42.	1	97.5	20	54.5	22	90.	5	52.
1902	22	90.	11	26.	3	85.	6	38.	14&27	90.	1	53.	31	90.	13	47.
1903	19	89.	2	**24.**	30	86.5	1	39.	9	94.	15	50.	18	85.5	8 & 14	45.
1904	25	88.	12	31.5	5–24 & 25	89.	12&17	45.	19	93.	3	49.	25	89.5	19	45.
1905	3	82.	2	29.5	19	90.	1	40.	18	92.	22	48.5	10	93.	27	41.
1906	24	88.5	11&21	30.	8	92.	12	37.	20–22 & 23	89.	25	50.	5	93.	16	47.
1907	14	85.	2–11 & 12	28.	18	94.	3	41.	16	90.	4	46.	12	96.5	19	41.5
1908	29	90.	1–4&5	31.	19	92.	12	43.	6–11 & 30	94.	9	52.	4	95.	25	46.
1909	31	78.	2 & 3	33.	28	90.	8	43.	15	92.	4	42.	8	98.	31	42.
1910	20	79.	16	31.5	22	89.9	4	**36.**	9	96.5	5	50.	3 & 15	90.	27	44.
1911	22	97.	3	27.	11	90.5	17	46.	5	**106.**	25–26 & 27	50.	8	94.5	30	47.
1912	24	88.	14	34.	1	89.	8	40.	8 & 10	95.	1	41.	14	92.	17	44.
1913	4	91.	11	30.	30	92.	9	37.	1 & 4	95.	12	50.	17	98.	25&26	44.
1914	26&27	90.	1	34.	24&25	90.	20	39.	11	93.	4	50.	9	94.	26	47.
1915	23	77.	3 & 4 10&27	34.	18&19	86.	4 & 9	41.	31	90.	22&23	49.	1	89.	27	48.
1916	24&28	82.	10&19	37.	27	82.	1	41.	30	96.	1	49.	22	101.	2 & 29	46.
1917	19	82.	4	32.	13&19 & 20	83.	16&17	46.	31	96.	4	50.	1 & 2	96.	31	47.
1918	6–18 & 31	84.	5	35.	1 & 2	90.	20	38.	21–22 & 28	95.	3	48.	13&14	98.	18&19	45.
1919	31	86.	6	32.	3	**96**	29	42.	4 & 5	95.	11	49.	7 & 8	92.	10	45.

* Data from record kept by Mr. Edgar Parker for the year 1895; Station record not available.

MONTHLY MAXIMUM AND MINIMUM TEMPERATURES FROM 1883 TO 1919, INCLUSIVE (*concluded*).

(Highest and Lowest Record for Each Month in Heavy Type.)

Year	September				October				November				December			
	Max.		Min.		Max.		Min.		Max.		Min.		Max.		Min.	
	Date	Temp.	Date	Temp.	Date	Temp.	Date	Temp.	Date	Temp.	Date	Temp.	Date	Temp.	Date	Temp.
1883	17	80.	11	37.	11	78.	17&18	25.	22	70.	17	13.	9 & 14	56.	23	—7.5
1884	5	91.	14	26.	5	84.2	27	23.	11	63.	25	15.	31	55.5	20	—15.5
1885	27	83.7	24	40.	1	79.	13	25.	8 & 13	68.	28	18.	24	53.	9	4.
1886	11	89.5	22	49.	10	76.7	17	27.5	3	68.2	28	17.	11&25	46.	6	—6.
1887	22	81.7	27	37.2	9	78.5	31	21.2	28	68.	30	15.	12	54.7	2	—3.
1888	1 & 10	83.	7	40.	6	62.7	22	29.	1 & 3	73.	23	8.	27	58.	22	4.
1889	4	84.	22 & 23&29	40.	2	68.7	24	21.2	4	61.7	17	17.8	25	60.5	4 & 5	8.
1890	8	83.6	25	35.5	5	69.8	31	32.	8	65.4	28	17.	1	46.2	20	8.
1891	26	92.8	30	43.	4	89.4	12&25	27.	1	68.	29	12.	5	57.7	18	7.
1892	26	88.	20	39.	1	82.	2	33.1	19	60.	24	18.	9	49.2	27	—8.7
1893	5	80.	26	37.4	13	76.	31	25.	3	62.	27	19.	26	62.	14	1.5
1894	4	90.	26	33.	1	76.5	15	33.	3	65.	29	12.	17	59.	29	—0.2
1895*	4	94.	15&30	42.	2	72.	30	28.	7	68.	21	19.	20&21	69.	13	—2.
1896	12	95.	23	36.	30	77.5	10&19	29.	19	70.	21	19.5	14	58.	28	2.
1897	11	98.	21	37.5	16	88.	10&18	30.	6	65.	24	16.5	12	61.5	24	2.
1898	4	94.	21	40.5	1	85.5	28	31.	5	63.	28	16.	31	54.	14	3.
1899	4	92.	15&30	30.	15	86.	3	26.	19	60.	14	25.	12	60.	31	—1.
1900	12	95.	19	37.	6 & 7	89.	20	28.	22	70.	17	19.	4	55.	10&14	4.
1901	6	89.	26	36.	10&11	74.	28	28.	1	65.	27	13.	14	62.	18	—1.
1902	1	90.	15	38.	19	74.	10 & 22&30	30.	14	73.	29	22.	2	52.	9	—5.
1903	14	90.	29	35.	1	73.	25&27	28.	4	70.	26&27	12.	3	46.	19	—4.
1904	3	88.	23	33.	10	81.	31	22.	3	65.	29	9.	23	53.	16	—2.
1905	30	88.5	26	36.	1	85.	26	20.5	12	61.	14	11.	29	52.5	15	1.
1906	18	91.5	25	38.	5	79.5	13&31	30.	19	62.	30	16.	6	52.	8	—1.
1907	20	90.	27	39.	†3	80.	31	24.	1	59.	12&16	22.	30	57.	22	13.5
1908	10	92.	30	37.	18	83.	21	27.	26	68.	5	18.	1	64.	23	3.
1909	14	93.	2 & 6	35.	9	82.5	29&30	27.	11	75.	24	21.	6	45.	30	1.
1910	6	87.	15&23	40.	6	81.	30	26.	1	59.	23	21.	29	41.	31	—2.5
1911	2	87.	14	35.	4	78.	3	33.	11	68.	13	18.	9	67.9	4& 30	13.
1912	6 & 10	95.	30	34.	6	83.	16	31.	6 & 7	69.	28	20.	6	65.	9 & 12	12.
1913	3	95.	15	28.	6	81.	31	29.	22	73.	27	22.	7	56.	28	6.
1914	22	92.	29	38.	10	84.	27	26.	1	69.	24	16.	2	64.	26&27	—6.
1915	14	93.	28	35.	13	75.	25	29.	1	68.	18	21.	25	45.	31	4.
1916	7	93.	30	39.	8	87.	27	29.	8	74.	25	16.	5	62.	30	4.
1917	1 & 2	82.	23	35.	2 & 18 19&30	64.	10	26.	11	58.	27	9.	24	44.	30	—18.
1918	2	82.	11	31.	29	78.	19	29.	15	65.	26	20.	14	55.	7	9
1919	7	92.	27	37.	19	29.	8 & 13	30.	1	71.	28	31.	13	50.	18	—6

* Data from record kept by Mr. Edgar Parker for the year 1895; Station record not available.
† Thermometer broken on the 27th, 28th, and 29th of October.

YEARLY MAXIMUM AND MINIMUM TEMPERATURES FROM 1883 TO 1919, INCLUSIVE.

(Highest and Lowest Record for THE TIME in Heavy Type.)

YEAR	MAXIMUM FOR EACH YEAR		MINIMUM FOR EACH YEAR	
	Date	Temp.	Date	Temp.
1883	Aug. 23	92.	Jan. 11	— 9.
1884	Aug. 20	95.	Dec. 20	—15.5
1885	July 18	90.5	Feb. 11	—11.5
1886	July 7	95.	Jan. 13	—18.7
1887	July 3	95.5	Jan. 19	— 8.
1888	June 23	94.1	Feb. 10	— 7.
1889	May 18	91.8	Feb. 4 and 24	— 7.
1890	Aug. 4	96.2	Mar. 8	2.
1891	June 16	95.	Feb. 15	2.5
1892	July 29	96.3	Jan. 10	— 5.
1893	July 26	95.5	Jan. 11	— 6.
1894	July 21	97.	Feb. 27	— 8.5
1895*	June 3	96.	Feb. 8	—14.
1896	Aug. 6 and 7	96.	Feb. 17	**—21.**
1897	Sept. 11	98.	Jan. 20	— 3.5
1898	July 4	96.5	Jan. 30 and 31	— 4.
1899	July 4 and Aug. 20	97.5	Feb. 11	— 8.
1900	Aug. 1	97.	Feb. 27	0.
1901	July 1	97.5	Feb. 24	2.5
1902	May 24, July 14 and 27, August 31 and Sept. 1	90.	Dec. 9	— 5.
1903	July 9	94.	Feb. 18 and Dec. 19	— 4.
1904	July 19	93.	Feb. 16	—18.
1905	Aug. 10	93.	Feb. 5 and 14	— 6.
1906	Aug. 5	93.	Feb. 6 and 7	— 7.
1907	Aug. 12	96.5	Jan. 24	—18.
1908	Aug. 4	95.	Jan. 2 and 5	—14.
1909	Aug. 8	98.	Jan. 19	— 7.
1910	July 9	96.5	Jan. 5	— 8.
1911	July 5	**105.**	Jan. 5	— 1.
1912	Sept. 6	95.	Jan. 14	—12.
1913	Aug. 17	98.	Feb. 10	—10.
1914	Aug. 9	94.	Feb. 13 and 24	—14.
1915	Sept. 14	93.	Jan. 30	— 3.
1916	Aug. 22	101.	Feb. 15	— 8.
1917	July 31, Aug. 1 & 2	96.	Dec. 30	—18.
1918	Aug. 13 & 14	98.	Feb. 5	—11.
1919	June 3	96.	Dec. 18	—6.

* Data from record kept by Mr. Edgar Parker; Station record not available.

MONTHLY AND YEARLY MEANS OF TEMPERATURES SINCE 1883.

YEAR	JAN.	FEB.	MARCH	APRIL	MAY	JUNE	JULY	AUGUST	SEPT.	OCT.	NOV.	DEC.	YEARLY AVER'GES
1883	17.4	22.3	28.6	43.3	52.0	66.6	67.4	65.6	56.3	46.6	39.1	27.5	44.0
1884	17.6	28.3	29.5	40.7	54.3	67.1	66.5	69.9	65.2	50.5	36.5	27.2	46.1
1885	20.6	11.4	18.8	41.2	54.3	63.6	69.7	65.0	58.3	49.2	39.8	27.8	43.3
1886	19.6	22.9	30.2	48.1	55.7	64.0	68.0	67.5	61.8	49.6	36.8	22.2	45.5
1887	20.2	23.2	26.3	41.1	62.5	65.7	75.6	66.5	57.7	47.0	37.6	27.6	45.9
1888	16.4	22.8	24.6	40.8	54.3	66.5	66.8	68.0	62.2	43.9	39.4	29.3	44.6
1889	29.1	18.1	33.9	45.1	58.4	65.3	70.2	66.0	60.5	44.0	40.3	35.2	47.2
1890	31.2	30.9	28.8	44.2	52.3	67.1	69.5	67.7	60.1	49.3	37.6	21.4	46.7
1891	25.9	28.3	30.8	45.3	52.0	66.4	66.4	68.5	66.2	48.3	38.4	35.5	47.7
1892	21.4	25.9	26.5	43.5	52.8	68.6	70.2	69.4	61.2	50.0	35.9	25.2	45.9
1893	15.5	20.6	29.5	41.1	54.1	68.2	69.8	68.8	58.0	52.0	38.2	27.5	45.3
1894	29.7	20.6	38.9	44.1	55.5	67.8	74.2	66.8	64.9	52.7	36.0	31.5	48.6
1895	21.8	16.9	26.9	44.4	59.0	......	......	71.2	61.7	45.4	39.6	31.4	....
1896	22.4	24.1	24.4	49.3	62.0	65.9	71.4	70.0	60.2	56.5	42.9	27.1	48.0
1897	23.2	26.1	33.8	45.0	55.4	62.3	73.6	67.6	62.3	52.6	39.7	29.2	47.6
1898	26.2	26.8	......	43.2	57.0	67.7	74.2	71.0	65.9	52.1	37.9	27.9	....
1899	22.1	20.4	30.4	46.6	57.6	69.5	71.2	71.6	60.6	53.5	38.9	30.0	47.7
1900	26.0	22.6	23.6	43.5	56.7	68.4	72.6	74.1	66.1	57.9	41.1	28.7	48.4
1901	26.1	18.5	32.2	46.5	56.9	68.9	76.6	71.0	64.0	51.4	34.3	27.7	47.9
1902	23.2	22.2	39.5	46.6	56.1	63.2	71.2	67.6	53.0	43.1	46.3	25.7	47.4
1903	25.7	28.1	42.2	45.9	60.4	63.2	70.8	65.5	64.4	52.5	36.2	23.3	48.2
1904	18.9	23.1	30.9	41.4	60.3	67.8	70.0	68.2	61.9	48.4	36.9	22.5	45.9
1905	19.8	18.9	33.1	44.8	57.5	66.4	71.8	68.7	63.7	52.4	37.6	32.0	47.2
1906	32.5	26.1	27.6	46.4	57.5	68.2	71.4	72.8	67.3	51.2	37.9	26.1	48.8
1907	24.9	19.5	38.1	40.2	51.3	64.0	71.2	68.4	64.4	47.9	38.7	31.8	46.7
1908	25.9	21.3	34.6	44.8	59.2	68.8	73.4	68.8	67.0	52.9	40.0	29.2	48.8
1909	27.7	28.6	31.0	44.3	57.9	67.2	69.6	70.0	63.5	47.7	44.5	25.7	48.1
1910	25.1	22.1	42.1	50.1	54.9	65.2	73.1	69.0	63.2	53.1	35.7	21.5	47.9
1911	24.9	26.6	30.9	44.8	64.9	67.5	74.4	70.9	62.8	50.7	36.6	35.1	49.4
1912	15.9	21.6	28.2	45.1	58.9	64.3	73.2	68.6	68.4	53.5	42.5	33.9	47.8
1913	32.7	18.8	36.9	48.3	56.9	66.8	72.1	70.0	61.3	54.0	44.5	33.6	49.7
1914	25.4	17.7	31.7	43.4	60.2	67.3	59.9	70.5	61.4	55.4	39.2	26.0	46.5
1915	26.5	29.6	31.0	52.3	52.6	64.6	69.6	67.8	65.6	52.6	41.1	28.5	48.5
1916	29.0	19.5	27.5	49.5	59.5	61.5	72.5	73.5	66.0	58.0	45.0	33.0	49.5
1917	24.6	20.2	33.8	45.8	49.5	63.9	71.8	70.5	60.1	45.9	35.3	19.5	45.0
1918	14.8	23.4	37.6	45.6	61.9	68.2	70.2	72.2	57.4	53.5	22.1	33.6	46.3
1919	31.0	29.2	35.7	43.6	57.0	71.8	72.0	67.8	64.7	55.4	39.6	23.4	49.3
AVERAGES	23.8	22.6	31.2	44.9	55.9	66.2	70.8	60.9	62.4	50.8	38.6	28.2	47.2

PRECIPITATION BY RAINFALL ONLY BY MONTHS SINCE 1882.

YEAR	JAN.	FEB.	MARCH	APRIL	MAY	JUNE	JULY	AUGUST	SEPT.	OCT.	NOV.	DEC.	TOTAL
	Inches	Inches	Inches	Inches	Inches	Inches	Inches	Inches	Inches	Inches[1]	Inches	Inches	Inches
1882	......	......	......	......	......	3.69	2.42	2.37	1.25	0.62	1.22	0.55	......
1883	0.48	1.44	0.88	1.58	4.45	4.12	2.98	3.47	2.12	2.10	1.54	0.73	25.89
1884	1.83	2.01	2.54	0.83	2.49	2.01	2.33	1.44	3.17	1.67	1.01	0.97	22.30
1885	1.07	0.61	0.12	1.26	1.58	2.49	4.64	5.02	2.11	2.88	1.36	0.76	23.90
1886	1.13	0.95	1.13	4.13	1.92	2.92	4.41	2.86	2.31	1.79	3.48	1.24	27.87
1887	0.18	2.97	0.48	1.37	0.46	2.01	6.37	3.03	0.75	1.74	1.58	1.35	22.29
1888	0.78	1.04	1.43	3.09	2.79	2.88	0.99+	4.02	2.73	3.47	2.02	1.24	20.48
1889	2.99+	0.25	0.66+	3.28	1.21	7.47	4.57	1.98	2.50	3.32	3.44	1.62	32.28
1890	2.16	1.45	2.16	2.20	5.49	4.26	1.07	4.34	5.81	4.54	2.40	......	36.88
1891	1.44	1.57	3.25	1.63	0.49	4.31	3.52	3.16	0.47	2.65	0.74	3.29	27.52
1892	0.57	0.88	0.55	0.67	4.04	3.95	1.89	4.77	1.12	1.34	1.67	0.72	23.17
1893	1.62	3.71	1.94	2.59	4.92	3.08	2.68	5.28	2.68	1.59	1.09	1.56	33.84
1894	2.21	2.71	1.36	2.43	7.03	1.77	1.50	1.22	4.64	3.59	0.43	0.47	29.36
1895	0.96	......	0.29	1.36	2.88	......	......	2.66	0.94	0.72	2.31	2.49	......
1896	1.19	2.28	0.84	0.41	2.31	3.71	4.12	3.33	4.27	2.26	2.18	0.71	27.61
1897	0.64	0.21	2.12	1.90	2.19	3.16	5.28	1.27	2.36	0.73	2.53	1.39	23.78
1898	1.74	0.33	1.54	2.03	1.90	2.37	1.32	3.60	1.86	3.83	2.03	0.33	22.90
1899	0.37	0.30	1.22	1.12	1.69	1.71	4.15	1.05	2.23	2.69	1.36	1.46	19.35
1900	1.43	2.42	0.02	0.95	1.71	1.45	6.53	1.75	0.91	3.65	6.13	0.78	27.73
1901	0.72	......	2.19	4.48	3.80	2.07	3.97	5.52	2.46	1.35	2.09	3.37	31.97
1902	0.86	0.66	1.94	1.92	2.84	4.33	5.29	2.41	2.88	2.32	0.74	0.74	26.89
1903	1.81	1.11	5.62	2.60	0.23	7.77	4.86	7.21	1.30	4.19	1.63	0.38	38.69
1904	0.80	1.08	2.41	1.67	4.04	3.37	5.73	2.56	3.26	2.06	0.26	1.42	28.61
1905	0.40	0.27	1.09	2.05	2.01	3.78	3.59	5.44	1.90	3.69	1.32	1.84	32.38
1906	1.46	0.53	1.60	2.06	4.24	5.31	2.37	3.68	2.16	3.56	1.40	1.54	29.93
1907	1.89	0.03	1.14	2.42	1.82	2.34	2.86	1.35	2.73	2.48	2.78	1.89	24.73
1908	0.68	1.12	1.24	3.28	3.57	1.96	4.72	1.79	1.66	2.73	0.88	0.43	24.06
1909	0.94	1.68	1.35	3.20	2.83	2.17	2.04	2.21	2.22	1.18	0.56	0.49	20.87
1910	0.87	0.53	0.28	4.56	3.45	1.55	2.39	5.47	3.29	1.73	0.62	0.38	25.12
1911	0.91	0.24	1.07	3.24	1.36	2.51	4.49	3.36	3.21	2.37	1.41	2.06	26.25
1912	0.20	0.95	1.92	3.41	7.27	2.09	4.85	2.21	5.89	1.42	1.48	1.13	32.82
1913	3.38	0.11	4.64	3.40	2.68	3.24	2.03	1.65	2.64	4.03	2.41	0.77	31.48
1914	0.96	0.15	1.28	3.10	4.00	2.69	2.13	6.05	1.62	1.55	1.06	1.13	25.81
1915	2.34	1.77	0.30	0.64	2.41	2.57	2.44	5.90	1.78	4.44	1.93	1.23	27.75
1916	2.69	1.05	0.50	3.40	5.69	5.83	1.78	3.47	2.15	1.41	1.83	1.49	31.29
1917	0.72	0.95	1.57	2.92	3.74	7.07	3.16	1.99	1.82	4.37	0.36	0.46	29.13
1918	0.19	0.91	2.09	2.08	4.10	3.38	4.21	2.25	3.60	3.39	2.25	1.78	30.23
1919	0.38	0.53	2.85	4.04	6.52	2.47	3.52	3.58	1.00	3.73	2.47	0.76	31.85
AVERAGES	1.25	1.14	1.60	2.30	3.23	3.58	3.56	3.38	2.48	2.63	1.78	1.24	28.30

TOTAL PRECIPITATION, RAINFALL AND SNOW REDUCED TO EQUIVALENT RAINFALL, 1918–1919.

YEAR	JAN.	FEB.	MARCH	APRIL	MAY	JUNE	JULY	AUGUST	SEPT.	OCT.	NOV.	DEC.	TOTAL
	Inches	Inches	Inches	Inches	Inches	Inches	Inches	Inches	Inches	Inches	Inches	Inches	Inches
1918	1.74	1.59	2.82	2.38	4.10	3.38	4.21	2.25	3.60	3.39	2.25	2.68	34.39
1919	0.78	0.93	4.15	4.34	6.52	2.47	3.52	3.58	1.00	3.73	2.67	1.91	35.60
AVERAGES	1.26	1.26	3.99	3.36	5.31	2.93	3.87	2.91	2.30	3.56	2.46	2.30	35.00

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